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"Fit to Fly" Biomarkers after Severe Traumatic Brain Injury with or without Additional Severe Trauma ("Multi-Trauma")

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14. ABSTRACT

Identification of prognostic adverse outcomes after traumatic brain injury (TBI) could preclude immediate long distance military air evacuation and determine if a patient is "Fit-to-fly" to a neurosurgical-capable facility. This study aimed to test models of various data sources and biomarkers to predict adverse intracranial pressure (ICP) changes in severe TBI prior to occurrence. Patients with severe TBI were prospectively enrolled. Continuously measured VS and biomarker levels were obtained on admission and every 6 hours for 72 hours. Systemic vital signs, such as blood pressure and heart rate, and intracerebral monitoring, such as ICP and cerebral perfusion pressure, were recorded. Multimodal statistical analysis including biomarker variable importance ranking was utilized to predict the next 6 hour outcomes of ICP >30mmHG for >15 minutes. By studying multiple cytokines with ICP we were able to identify a combination of biomarkers, with statistical methods of variable ranking to determine a strong relation to neurological worsening.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

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1.0 SUMMARY

Identification of prognostic adverse outcomes after traumatic brain injury (TBI) could preclude immediate long-distance military air evacuation and determine if a patient is “Fit-to-fly” to a neurosurgical-capable facility. This study aimed to test models of various data sources and biomarkers to predict adverse intracranial pressure (ICP) changes in severe TBI prior to occurrence. Patients with severe TBI were prospectively enrolled. Continuously measured VS and biomarker levels were obtained on admission and every 6 hours for 72 hours. Systemic vital signs, such as blood pressure and heart rate, and intracerebral monitoring, such as ICP and cerebral perfusion pressure (CPP), were recorded. Multimodal statistical analysis including biomarker variable importance ranking was utilized to predict the next 6-hour outcomes of ICP >30mmHG for >15 minutes. By studying multiple cytokines with ICP we were able to identify a combination of biomarkers, with statistical methods of variable ranking to determine a strong relation to neurological worsening.

2.0 INTRODUCTION

The prospective study is the investigation of the efficacy of selected neurotrauma biomarkers in predicting critical adverse events in patients with severe TBI at least 12 hours before these events. Long-distance air evacuation of the most seriously injured casualties is a central feature of modern casualty care. Determining the optimal “fit to fly” time in the first 72 hours after admission to Level III care is therefore a priority, but evidence on which to base such protocols is scant, particularly in casualties with severe TBI. Previous work at our center into inflammatory cytokines and other biomarkers of severe neurotrauma suggests that, using selected neuro-trauma biomarkers present in serum, we can predict critical adverse events in patients with severe TBI at least 12 hours before these events. In this single-center observational study, we assessed the power of selected serum biomarkers to predict, in 6-hour intervals, adverse outcomes after severe TBI and in patients with severe TBI and poly-trauma. All work was carried out at the University of Maryland sites. Including the University of Maryland School of Medicine and its associated R Adams Cowley Shock Trauma Center (STC) and Shock Trauma and Anesthesia Research Organized Research Center (STAR-ORC).

Long-distance air evacuation of seriously injured casualties, after preliminary stabilization, to Level IV care in Europe and then to Level V care in the Continental United States inevitably imposes additional physiologic stresses and provides limited scope for monitoring and intervention. Determining the optimal “fit-to-fly” time in the first 72 hours after admission to Level III care is, therefore, a priority, but evidence on which to base such protocols is scant, particularly in casualties with severe TBI. TBI is a leading cause of death and persistent disability in the current conflicts, and casualties with severe TBI (Glasgow Coma Scale—GCS—score of 8 or less) are particularly vulnerable to these stressors. Clinical research at our center into inflammatory cytokines and other biomarkers of severe neurotrauma suggests that, using selected neurotrauma biomarkers, we could predict critical adverse events in patients with severe TBI at least 12 hours before these events.

We proposed by expand this work with the specific aim of assessing the power of serum levels of these biomarkers to predict, in 6-hour intervals, adverse outcome after severe TBI and in patients with severe TBI and poly-trauma. This work intention is to provide the basis for eventual fielding of valid, reliable, and robust and useful point-of-care testing as adjuncts to “Fit

to fly" air-evacuation decision-making. For study course refer to individual reports and reference addendum 1.0 for detailed Institutional Review Board (IRB) and study adjustments.

The study addresses a key area and knowledge gap of the En Route Care Modernization Thrust Area: patient staging and en route patient safety (III.1.3). TBI is the most common cause of death and long-term disability in combat casualties, and long-distance air evacuation is a critical component in current management strategies for these patients. However, as noted above, the evacuation itself is not without specific risks for additive injury. The ability to fine-tune evacuation times to the needs of individual patients, within the first 72 hours after injury, will improve long-term outcomes for these patients. Once the predictive patterns of serum biomarkers that reflect impending intracranial insults after severe TBI in the setting of multi-trauma are determined, the next stage in research will include development of appropriate point-of-care test panels for the specific biomarkers identified.

3.0 BACKGROUND

Modern combat casualty care is centered on rapid evacuation, mostly by air, from the battlefield through increasingly sophisticated levels of trauma care. Once the severely injured are stabilized in-theatre at a Level III combat support hospital, they typically face two long-distance evacuations, the first to Level IV care in Europe and the second to Level V care in Continental United States. These flights are themselves physiologically stressful and over their duration offer limited scope for interventions. Determining the optimal "fit-to-fly" time in the first 72 hours after admission to Level III care is, therefore, a priority, but evidence on which to base such protocols is scant, particularly in casualties with severe TBI. TBI is an important cause of death and persistent disability in the current conflicts, and individuals with severe TBI (GCS score of 8 or less) are particularly vulnerable to the stressors of long-distance air evacuation [1-5]. Previous work at our center has shown that serum levels of the inflammatory cytokine interleukin 8 (IL-8) and tumor necrosis factor-α (TNF-α), associated with central nervous stem damage and inflammation, can predict cerebral hypoperfusion and intracranial hypertension in patients with isolated severe TBI with a lead time of 12 hours [6,7]. In addition, we found that levels of the known markers of neuronal cell death, S-100-beta (S100-β), a calcium binding protein, and neuron specific enolase (NSE), correlated with impending cerebral hypoxia [8]. We expanded this work to assess levels of these biomarkers in individuals with severe TBI over 6-hour time intervals and in individuals with multiple injuries. The shorter time intervals are more reflective of the need for decision-making support of the front-line clinician, and severe TBI that includes multi-trauma is more reflective of both the decision-making needs and the physiologic situation in the care of combat casualties.

Our hypothesis in this work was that the serum biomarkers that we have identified as significant predictors in isolated TBI over the first 12-hour intervals after injury for cerebral hypoperfusion (CH), cerebral hypoxia (CHx), and intracranial hypertension (ICH) will demonstrate the same predictive power after multitrauma that includes severe TBI and will be demonstrable over shorter time periods.

3.1 Specific Aims and Experimental Design

The overall aim of this study was the investigation of the power of inflammatory cytokines and markers of neuronal cell death to predict episodes of CH, CHx, and ICH earlier

and at shorter intervals over the first 3 days after injury than previously described and in a patient-injury population more consistent with combat casualties. Our long-term goal in this work is the fielding of robust and efficacious point-of-care test modalities as adjuncts to "fit-to-fly" air evacuation decision-making.

Our study is a single-center, prospective, observational study with the following specific aim and objectives:

Aim: To assess the power of serum levels of four biomarkers—IL-8, TNF-α, S100-β, and NSE—to predict clinically significant episodes of CH, CHx, and ICH, as measured by CPP, ICP, and PbO_2 (partial pressure of oxygen in the brain) beginning within 6 hours of injury and over 6-hour periods subsequently over the first 72 hours of care at the STC of the University of Maryland School of Medicine (UMDSOM) for both isolated severe TBI, that is, severe TBI not associated with significant injury to other body systems, and severe TBI that is associated with significant additional traumatic injuries—a situation more consistent with combat injuries.

Objectives: Secure UMDSOM and United States Air Force IRB approval for this study. For each eligible patient, secure informed consent from the Legally Authorized Representative (LAR) within 6 hours of injury.

Enrollment of 100 adult (older than 17 years) patients admitted to the STC within 6 hours of injury, severe TBI as defined by Head AIS>2 and post-resuscitation mGCS<6, Isolated TBI with or without additional injury as defined by at least one other body region with AIS>3 or two body regions with an AIS=2 and who have placement of a clinically indicated ICP monitor within the first 6 hours. Exclusion criteria are nonsurvivable TBI or monitoring begun >6 hours post-injury. TBI will be confirmed by computed tomography (CT) and Marshall Classification Scores will be assigned by a blinded reviewer, as in our previous study [6]. The sample size was chosen based on our previous work in which 50 patients were found to produce both clinically and statistically robust findings in patients with isolated TBI. All admissions to the Trauma Center will be screened for possible enrollment. About 350 patients with severe TBI will be screened in more detail and based on our inclusion and exclusion criteria, as well as our consent and LAR availability rates in experience with previous studies, we predict that 100 patients (50 with isolated TBI and 50 with multitrauma and TBI) is entirely reasonable and feasible. TBI patients at the STC are managed according to an institutional protocol based on Brain Trauma Foundation (BTF) Guidelines [9] (See also below, Section 11 – Capabilities). Pertinent patient demographic, clinical, general laboratory, and study sample laboratory data will be assembled and stored in password-protected databases in dedicated systems.

Upon admission and every 6 hours at standard times over the first 72 hours of admission, draw and prepare serum samples for analysis for IL-8, TNF-α, S100-β, and NSE. The laboratory infrastructure to perform this testing is already in place, but test materials will have to be secured. Blood is drawn into standard 5-mL serum collection tubes, centrifuged to remove any cellular debris and then frozen at minus 80°C until batch processing to maximize efficiency of the Luminex® (Luminex Corp., Austin, TX) system and utilization of the Millipore® (Millipore, Billerica, MA) assay kits. Analytical limits of detection for each of the biomarkers of interest have been established [6]. All samples are run in duplicate. Test result data are recorded electronically and stored securely and will be correlated by dedicated study research staff in the secure study database.

Monitor all individuals enrolled with the continuous electronic automated vital signs visualization and data collection capacity currently available in our Neuro-Trauma Critical Care Unit. The monitoring data accrued will be segregated and stored securely for linkage with

demographic, injury-specific clinical, imaging, general laboratory, and study-test-sample laboratory data for subsequent analysis. All of these collection, storage, retrieval, and processing systems are in place and functioning at this time. Data include continuous real-time assessment of conventional vital signs and physiologic parameters—SpO_2, etc.—but also ICP, CPP, pressure-times-time of ICP and CPP, shock index (systolic blood pressure divided by heart rate), and Brain Trauma Index (CPP/ICP) [10-12].

Correlate serum biomarker levels over time with the results of continuous monitoring as above and with selected adverse outcomes in the interval between serum samples. These adverse outcomes include occurrence and duration of CH measured by CPP, ICH measured by ICP, CHx measured by PbO_2, and/or the need for surgical decompression. (See also below – Capabilities.) As noted above, vital signs and ICP monitoring data were recorded by automated systems as continuous raw data and also signal-processed into various indices of clinical relevance [10-13]. *Statistical analysis* included the use of Student's t-test to assess means based on continuous data assumed to be normally distributed. Continuous data that are not normally distributed will be assessed using the nonparametric Wilcoxson's rank-sum statistic. Linear regression methods were utilized to assess correlation between cytokine levels and ICP and CPP and PbO_2. A calculated probability (P value) of less than 0.05 that the results were due to chance and considered significant.

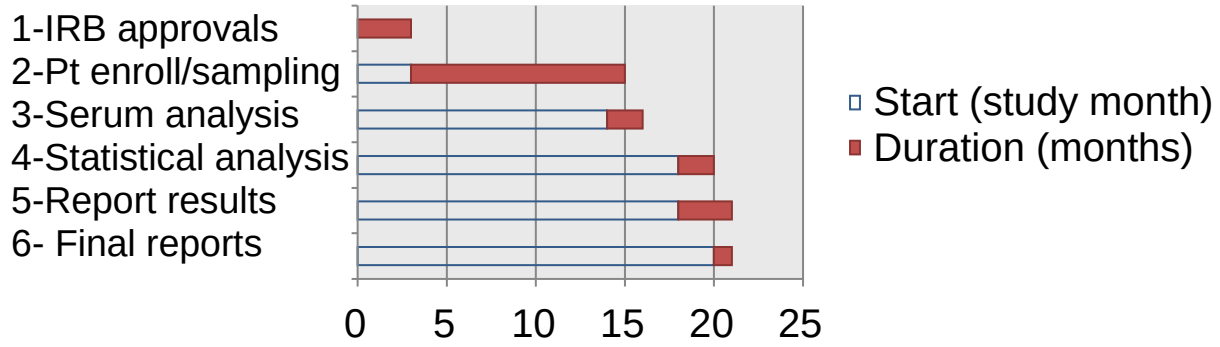
3.2 Milestones/Deliverables

This study had a specific aim, described in detail above. We anticipated completion of all administrative and scientific study objectives Table 1 shows these objectives expressed as technical milestones and deliverables, with their initial estimated durations and time periods Table 2)

Table 1. Milestones and Deliverables

Milestone		Deliverable	Duration	Timing
1	All IRB approvals (administrative)	demonstration of study feasibility	3 mo	mo 1 – 3
2	Complete patient enrollment (administrative)	demonstration of study feasibility	12 mo	mo 3 – 15
3	Complete serum sample analysis (scientific)	raw test data for statistical analysis	2 mo	mo 4 – 16
4	Complete statistical analysis (scientific)	P-values	2 mo	mo 16 –18
5	Scientific reports to appropriate peer-reviewed groups and journals (scientific);	abstracts and reports in submission	3 mo	mo 19 – 21
6	Complete all grantor reports (administrative)	all required reports	1 mo	mo 21

Table 2. Gantt Chart/Schedule



3.3 Risk Analysis and Alternatives

The infrastructure for the study, including laboratory testing capacity and trauma resuscitation unit (TRU) research personnel, experienced in assessing eligibility for this type of study and securing informed consent in a timely manner, already exist at our center. The study proposes no risks in excess of previously approved and successful work, and no testing systems currently exist that can circumvent the need for serum sampling. The main potential limitation of the study was patient accrual over time. Our Level 1 trauma center currently admits roughly 5,000 patients annually directly from the scene of injury (therefore within six hours of injury) of whom about 2,000 have some form of head injury roughly 350 of whom fit the above criteria for severe TBI [14]. We are also the regional referral center for adult neuro-trauma. For these reasons, we expected to be able to meet the patient-accrual projects in the planned study period.

3.4 Technical Program Summary

We proposed the assessment of the statistical power of serum levels of biomarkers known in isolated, severe TBI and at 6-hour intervals to predict clinically significant episodes of CH, CHx, and ICH—adverse events of critical importance to the survival and good functional outcome of combat casualties with severe TBI, events likely to be worsened by the conditions of long-distance air evacuation and for which long-distance air evacuation provides little scope for intervention—but beginning within 6 hours of injury and in the setting of multi-trauma, a time-frame and clinical scenario that would allow these markers to support “fit-to-fly” decision-making.

This work is a direct follow-on to successful preliminary work in this field:

- The preliminary work has already identified, among a number of potential biomarkers for adverse outcome in neurotrauma, the four leading candidates listed above.

- Both the UMDSOM and United States Air Force IRBs have approved the prior, very similar, study.

- The infrastructure for this project, including laboratory, monitoring, and major computer processing equipment, is already in place.

- Key research staff, including the front-line personnel who identify eligible patients, obtain informed consent, and gather and enter demographic and other clinical data, are already in place and experienced in this general area of research.

- Modalities and procedures for data collation, statistical analysis, and reporting are already in place and have demonstrated efficacy for a study protocol of this type.

- All of the above factors supported a highly efficient study that is likely to be completed within budget and within the proposed time frame.

3.5. Capabilities

3.5.1 Clinical Facilities. The STC, located at the University of Maryland Medical Center in downtown Baltimore, cares for over 8,000 injured patients each year. The STC is a free-standing dedicated trauma hospital and provides the highest level of care for critically ill and injured patients in the state as the Primary Adult Resource Center for Maryland's emergency medical services system. The STC is also the Specialty Referral Center for the State of Maryland for neurotrauma. As noted above, in 2010, approximately 2,000 patients with TBI were admitted to the STC. Of these, over 350 suffered a severe TBI. The STC has a dedicated 12-bed Neurotrauma Critical Care Unit that cares for more than 400 patients per year, associated with a 12-bed dedicated Neurotrauma Intermediate Care Unit. Patients are admitted directly from the scene of injury or in transfer from other hospitals exclusively through the 13-bed TRU. Computed tomography scan and MRI are available 24 hours per day. There are six dedicated trauma operating rooms (ORs), with the capacity to immediately accommodate any surgical emergency at any time of the day or night. We are currently in the process of expansion into a new facility that will further expand all of these capabilities.

As noted above, the management of neurotrauma patients is highly protocolized using an institutional algorithm based on the BTF Guidelines [9]. These guidelines outline clinical management strategies to maintain ICP at <20 mmHg and CPP at >60 mmHg, including sedation; analgesia; mechanical ventilation to maintain $PaCO_2$ of 35-40 mmHg; head elevation (30°-45°); and maintenance of normal oxygenation, blood pressure, and volume status. In accordance with the BTF Guidelines, first-tier therapies of intracranial hypertension (ICH: ICP >20 mmHg) include insertion of external ventricular drainage via an intracranial catheter, increasing sedation, and/or hyperosmolar therapy [14]. Intractable ICH is treated with moderate hyperventilation ($PaCO_2$ <35 mmHg), induction of barbiturate coma, and decompressive craniectomy or laparotomy [15, 16]. Management of CHx, as measured by PbO_2, is also highly protocolized and evidence based [17]. Dedicated, integrated, and aggressive neurosurgical and neurotrauma critical care of these patients has resulted in exceptional results with significantly lower mortality rates than reported by most institutions. Routine functional outcome evaluations for these patient populations are also conducted per standard-of-care in the outpatient setting.

The Program in Trauma at our center is distinctive in that multiple disciplines are represented on a full-time basis, each devoting 100% of their clinical practice to the care of critically ill and injured patients at the STC. Divisions of Trauma Surgery, Trauma Critical Care

Medicine, Trauma Anesthesiology, Trauma Neurosurgery, Hyperbaric Medicine, Infectious Diseases, Orthopaedic Traumatology, Trauma Plastic Surgery, Trauma Radiology, and Wound Healing are each composed of fellowship or subspecialty-trained experts in these fields. Clinical coverage is provided all times/all shifts by in-house trauma-specialist attending physicians in surgery, critical care, radiology, and anesthesiology. In addition, full time in-house coverage is provided by two dedicated trauma critical care fellows; a trauma surgery fellow; and house staff in general surgery, emergency medicine, and anesthesiology. Trauma orthopedic coverage is provided by an in-house trauma orthopedics fellow and trauma orthopedics resident. Neurosurgical coverage is provided by an in-house senior trauma neurosurgery resident.

The STAR-ORC is the core of the Program in Trauma Research Enterprise. STAR-ORC coordinates the infrastructure for all research projects conducted at the STC. A dedicated staff of research nurses, research assistants, and research project coordinators are available in-house to provide all days/all shifts research staffing. All patients admitted to the TRU are screened by the research staff for possible inclusion in ongoing studies. The research staff determines eligibility for protocols, obtains informed consent, and provides study coordination and data collection. Additional research staff provides statistical support, regulatory document preparation, and post-award grants management. STAR-ORC has incorporated additional resources and expertise from the Charles McC. Mathias National Study Center. To date, more than 20 investigators have been recruited, both externally and from multiple departments within the UMDSOM, 12 of whom focus on acute brain injury, including Dr. Alan Faden, the medical director of STAR-ORC. Other important resources include multiple National Institutes of Health (NIH) pre-doctoral and post-doctoral training programs, multiple seminar series, and the proximity to the NIH and to the Johns Hopkins University School of Medicine.

The STC Processing Laboratory within the STC is equipped with state-of-the-art technology, including a So-Low® Premier freezer (So-Low Environmental Equip. Co., Inc., Cincinnati, OH) and Eppendorf® Refrigerated Centrifuge (Eppendorf, Hamburg, Germany) and is available to research staff at all times are centrifuged to specimen processing and storage of human samples. As described above, serum specimens remove cellular debris and the supematant immediately frozen at -80 Celsius until analysis.

Cytokine analysis for purposes of this study will be performed by STAR research personnel on-site. The Luminex® 200 System (Luminex Corp., Austin, TX) is a multi-analyte bioassay detection system capable of multiplexing up to 100 assays simultaneously in a single micro-titer plate well. This system combines internally colored microspheres, lasers, optics, fluidics, and advanced digital processing into a single, integrated system that increases assay specificity and throughput. The system is specifically designed for multiplexing a wide range of bioassays such as immunoassays, enzyme functions, receptors-ligand interactions genotyping, and HLA typing. It is specifically engineered to meet and exceed the need for in-vitro diagnostic requirements in clinical laboratories.

3.5.2 Trauma Center Real-Time Vital Signs Data Registry. Real-time patient vital signs data feed from 103 monitors (GE-Marquette-Solar-7000/8000®, General Electric, Fairfield, CT) are networked for the entire STC. A total of 103 patient-bed locations include 13 TRU, 6 OR, 2 angiography suite, 1 CT, 9 post-anesthesia care unit, 36 ICU and 36 intermediate care unit beds. Real-time vital signs waveforms, trends, and alarms are compressed and transferred to a centralized VSDR server through the secured hospital intranet and archived. We have developed custom processing and viewing programs (based on Matlab and VisualBasic) and use them for

real-time patient data abstraction, artifact removal, 5-60 min time window averaging, vital signs (VS) variability, and summary data output to both text and MS-SQL format. The server is interfaced with the Trauma Registry, which provides over 100 patient-specific demographics and outcomes. The real-time TRU and ICU VS viewer was developed to highlight critical episodes of VS and "dose" (pressure times time) for the previous 12 and 24 hours and 7 days. VSDR collects over 80 VS variables, including continuous electrocardiogram, oxygen saturation, end-tidal carbon dioxide (ECG/SpO_2/CO_2/respiration) waveforms at 240 Hz. The numerical values of heart rate, blood pressure, ICP, CPP, respiratory rate, and temperature are recorded every 6 seconds for all 103 patient beds. Raw data are compressed over 90% before being sent to the VSDR server. Data rates after compression averaged 76.4 KB/h for numerical and 12.3 MB/h for waveforms.

3.5.3 Equipment. Computer support: In addition to the data gathering equipment at the core of the VSDR, STAR-ORC has a full range of computing power, connected to a Novell Network allowing communication with the University of Maryland Medical Center and the School of Medicine, the CERNER Electronic Health Records, and Paxis radiological records (with laboratory clinical and radiological summaries and reports). STAR-ORC has access to the full range of clinical information generated by the STC, including archived vital signs data; live video feeds from critical clinical areas such as the TRU, ORs, and ICUs, including the neuro-trauma critical care unit; and all data captured in the Trauma Registry. The investigators have developed an automated Trauma Registry query system providing more than 100 diagnoses and outcomes for each patient admitted to the STC.

3.5.4 Relevant Experience. A broad range of funded, recent, ongoing, and pending research efforts forms the background to the proposed study, however, the most specific are those related to neurotrauma biomarkers vital signs signal processing. Many of these were funded through the U.S. Army Medical Research & Material Command: W81XWH-07-2-0118; Early Support of Intracranial Perfusion [$5,939,988]; (09/2007–10/2012).

4.0 METHODS

This prospective study was conducted at STC at the University of Maryland Medical Center. The study was approved by expedited review of IRB from the University of Maryland School of Medicine. The inclusion criteria were adult trauma patients (age≥18 years old) direct admissions with TBI (isolated or polytrauma) and admitted to Intensive Care Unit (ICU) for continuous physiologic monitoring (Refer to addendum 1.0 for changes to the inclusion criteria and quarterly reports indicating changes to the aim and design of study) Included patients had ICP monitor, either intraventricular catheters (IVC) or intra-parenchymal pressure monitors (Camino) placed within 24 hours of admission. Transferred patients, active cardiac arrest or mortality within 24 hours of trauma center arrival were excluded from the study. Further excluded were patients with non–survivable brain injury, mild TBI who did not require ICP monitoring and patients with missing data or demographic information. The study screened a total of 875 patients were screened that were admitted to the regional adult neurotrauma center/ level I trauma center, of whom 176 met all study eligibility criterial and 62 were enrolled. Continuously measured VS and cytokine levels (CYT) were obtained on admission and every 6 hours for 72 hours. Medication, SVS, such as blood pressure and heart rate, and ICM, such as

ICP and CPP, were recorded. Boosting decision trees were used to rank the importance of medication, SVS, ICM and CYT to predict four outcomes in the following 6 hours: (1) $ICP_{>20mmHg}$ > 30min, (2) $ICP_{>30mmHg}$ > 15min, (3) $CPP_{<60mmHg}$ >30min, (4) $CPP_{<50mmHg}$ >15min. Furthermore, in-hospital mortality and near-term neurologic deterioration were also considered as outcomes for comparing the usefulness of variables. Each patient dataset is linked to essential demographic information such as age and sex. Each patient dataset then includes 13 consecutive rows of measures of vital signs and cytokines, and each row is 6 hours after the preceding one. Therefore, each patient has a total span of 72 hours of vital signs and cytokines.

4.1 Data Sources and Feature Design

Multiple data sources were observed and recorded as potential patients who met the inclusion criteria were monitored at least 72 hours after admission to ICU (Figure 1). Approximately every 6 hours, a sample of blood was drawn and kept for cytokine and biomarker analysis. Blood samples were saved in a freezer and sent for analysis. Readings from blood samples include IFN-G, TNF-alpha, IL-2, 4, 5, 6, 7, 8, 10, 1B, 13, GMCSF, NSE, S100β. Automated electronic continuous VS were recorded, including invasive ICP and mini-invasive arterial blood pressure or non-invasive blood pressure, and HR. Medication information was also documented on hourly basis.

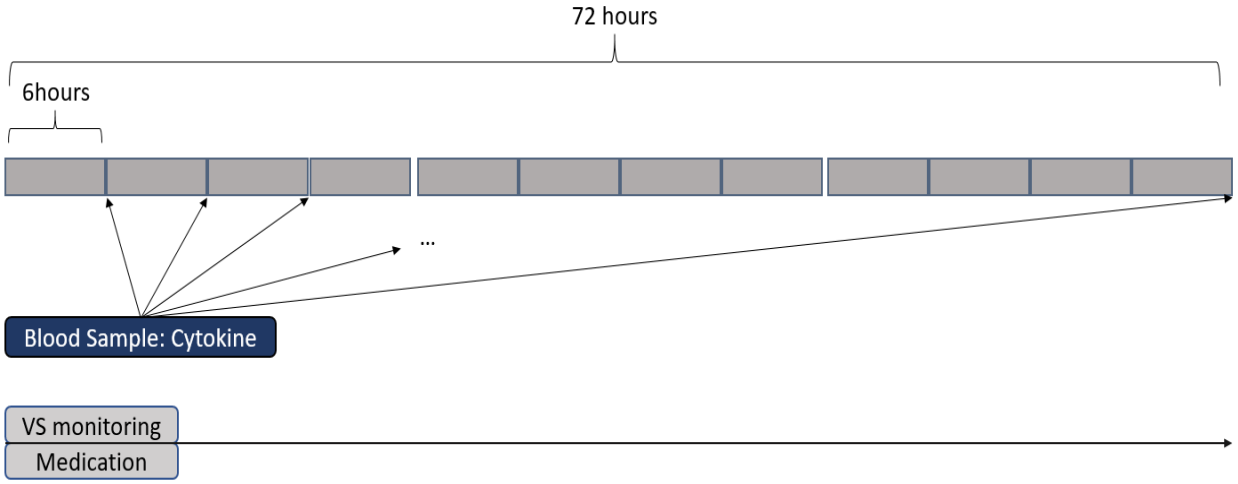


Figure 1. Data sources and data collection scheme. In a total 72-hour observation duration, a blood sample is drawn every 6-hour. Continuous VS and medication information are collected and aligned by time. The 6-hour window was later changed refer to Enrollment addendum 1.1.

For continuous VS, we used summary statistics to aggregate long time series into single variables that represent the patients' physiologic conditions. Maintaining patients' vital signs within normal ranges is a basic task for clinicians. Some treatment protocols also give guidelines on the thresholds of vital signs to be watched during patient care. For example, for the management of severe traumatic brain injury, it is recommended to initial treatment when ICP is above 20 mm Hg, and CPP is suggested to remain above 70 mm Hg [18]. The guidelines for field triage of injured patients recommended to use SBP< 90 mmHg or RR <10 or >29 breaths per minute as a part of the physiological criteria when considering if a patient should be transported to a facility with the highest level of care [19]. Those thresholds that pass muster

with the clinical experts indeed can serve as domain knowledge to design features from physiological time series. Such type of features may hold clinical meanings that are easy to be interpreted by humans. To quantify the cumulative effect of VS away from normal range, the "pressure times time does" (PTD) is defined as the integrated area enclosed by the VS curve and the threshold line within a given time interval. Sometimes, to compare between patients, it is also calculated as averaged PTD in unit time, which is the PTD normalized by time duration. Even though two patients may be monitored of different time durations, their PTD in unit time is still comparable. In predicting TBI patients outcome, the PTD of ICP>20 mmHg and CPP <60 mmHg have been shown to be good predictors of in-hospital mortality and length of ICU stay [40]. Other descriptive statistics of VS within a given time period are also informative for clinician use or may contribute to outcome prediction in a model. With the assumption that the observed data are approximately normal distributed, mean and variance are often used to sketch the VS value distribution. Standard deviation (SD) is used to quantify the variability of observed VS data. The coefficient of variance, which is the SD divided by the mean, is a unit-less value that suitable to compare between datasets with widely different means. Robust statistics, such as percentiles or quartiles, are also used to quantify the shape of the VS data distribution. Median (50 percentile or 2^{nd} quartile) is one of the most commonly used statistics in VS feature calculation.

Our hypothesis is that information derived from continuous automated recordings of patient VS, including ICP, SBP, DBP, medication, and sequential levels of 15 CYT known to be associated with adverse outcome after TBI can be used to predict the likelihood of future onset of adverse cerebral pressures within a time frame that would preclude immediate air evacuation. Our specific aims are to predict the following 4 outcomes in the 6 hours subsequent to the time frame of the predictive features. The outcomes include $ICP_{>20mmHg}$ > 30min, $ICP_{>30mmHg}$ > 15min, $CPP_{<60mmHg}$ >30min, $CPP_{<50mmHg}$ >15min, and for the next 6 hours after each blood draw.

To determine which features of the data may be more important in predicting the outcomes of interest, exploratory comparisons were used to compare difference between that group of patients whose outcome markers stayed within normal ranges and those whose outcome markers exceeded those ranges. The comparison tests included the two-sample t-test for mean difference comparison, the two-sample Kolmogorov-Smirnov test for distribution comparison, and the Wilcoxon rank sum test for median difference comparison, to compare the cases with positive outcomes and the cases with negative outcomes as shown in Tables 3, 4, and 5. Tests with p-value<0.05 is considered as significantly different. As we can observe from Tables 3-5, a general pattern is that variables calculated from invasive ICP or CPP often had statistical difference between the positive and negative outcome groups, which means that those variables potentially are good predictors to separate outcomes. Variables calculated from non-invasive or mini-invasive VS, such as SBP and HR often had no significantly difference between positive and negative outcome groups. Biomarker variables had shown some mixed pattern in significance tests. IL-8 appeared to have significant mean difference between outcome labels. However, not all biomarkers had consistent pattern in significance tests.

Based on above exploratory tests, we categorized the variables into four categories. Category 1 included invasive VS (such as ICP and CPP). Category 2 included non-invasive vital signs (such as MAP, SBP, DBP, HR), category 3 included cytokines, and category 4 were variables derived from medication.

Table 3. P-values of Two-Sample t-test for Mean Difference Comparison

Mean Difference Comparison	ICP_Time_20	ICP_time_30	ICP_Mean>15	ICP_Mean>20
pre6H_pct_ICPgt20	0.000	0.000	0.000	0.000
pre6H_pct_ICPgt30	0.000	0.000	0.000	0.000
pre6H_PTD_ICPgt20	0.000	0.000	0.000	0.000
pre6H1Q_ICP	0.000	0.000	0.000	0.000
pre6H2Q_ICP	0.000	0.000	0.000	0.001
pre6H3Q_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_ICP	0.000	0.000	0.000	0.000
pre6H_Min_ICP	0.000	0.520	0.000	0.586
pre6H_Max_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_CPP	0.003	0.000	0.000	0.003
pre6H_Min_CPP	0.024	0.000	0.003	0.005
pre6H_Max_CPP	0.007	0.000	0.001	0.057
pre6H_Mean_MAP	0.263	0.723	0.806	0.586
pre6H_Min_MAP	0.407	0.706	0.329	0.970
pre6H_Max_MAP	0.712	0.712	0.324	0.414
pre6H_stdSBP	0.385	0.911	0.205	0.812
pre6H_stdDBP	0.212	0.771	0.398	0.552
pre6H_stdHR	0.528	0.719	0.976	0.810
pre6H_Mean_HR	0.688	0.347	0.162	0.451
pre6H_Mean_SBP	0.491	0.484	0.564	0.587
pre6H_Mean_DBP	0.032	0.403	0.957	0.699
IFN-G	0.437	0.803	0.141	0.529
TNF-A	0.565	0.034	0.356	0.613
IL-2	0.587	0.759	0.294	0.321
IL-4	0.740	0.419	0.911	0.457
IL-10	0.405	0.418	0.122	0.045
IL-1B	0.671	0.410	0.601	0.971
IL-6	0.955	0.853	0.280	0.982
IL-8	0.951	0.016	0.015	0.000
GMCSF	0.286	0.970	0.561	0.764
IL12P70	0.758	0.285	0.665	0.620
IL13	0.627	0.583	0.578	0.611
IL5	0.037	0.498	0.185	0.893
IL7	0.363	0.525	0.522	0.091
NSE	0.856	0.713	0.453	0.359
S100B	0.157	0.529	0.571	0.658

Table 4. P-values of Two-Sample Kolmogorov-Smirnov Test for Distribution Comparison

Distribution Comparison	ICP_Time_20	ICP_time_30	ICP_Mean>15	ICP_Mean>20
pre6H_pct_ICPgt20	0.000	0.000	0.000	0.000
pre6H_pct_ICPgt30	0.000	0.000	0.000	0.000
pre6H_PTD_ICPgt20	0.000	0.000	0.000	0.000
pre6H1Q_ICP	0.000	0.000	0.000	0.000
pre6H2Q_ICP	0.000	0.000	0.000	0.084
pre6H3Q_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_ICP	0.000	0.000	0.000	0.000
pre6H_Min_ICP	0.006	0.688	0.000	0.714
pre6H_Max_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_CPP	0.006	0.000	0.000	0.001
pre6H_Min_CPP	0.102	0.024	0.023	0.001
pre6H_Max_CPP	0.011	0.001	0.001	0.070
pre6H_Mean_MAP	0.340	0.561	0.649	0.359
pre6H_Min_MAP	0.285	0.861	0.373	0.479
pre6H_Max_MAP	0.581	0.262	0.102	0.164
pre6H_stdSBP	0.132	0.266	0.088	0.434
pre6H_stdDBP	0.624	0.423	0.203	0.113
pre6H_stdHR	0.257	0.812	0.519	0.388
pre6H_Mean_HR	0.611	0.519	0.305	0.882
pre6H_Mean_SBP	0.658	0.857	0.581	0.355
pre6H_Mean_DBP	0.054	0.417	0.213	0.083
IFN-G	1.000	1.000	0.906	1.000
TNF-A	0.130	0.543	0.246	0.448
IL-2	0.895	0.074	0.735	0.988
IL-4	1.000	1.000	1.000	1.000
IL-10	0.368	0.046	0.098	0.030
IL-1B	0.999	1.000	0.986	0.738
IL-6	0.150	0.065	0.021	0.182
IL-8	0.255	0.069	0.176	0.018
GMCSF	1.000	0.958	0.244	0.165
IL12P70	1.000	1.000	1.000	1.000
IL13	1.000	1.000	1.000	1.000
IL5	0.468	0.245	0.454	0.092
IL7	0.762	0.925	0.254	0.391
NSE	0.458	0.358	0.032	0.357
S100B	0.668	0.948	0.980	0.382

Table 5. P-values of Wilcoxon Rank Sum Test for Median Difference Comparison

Median Difference Comparison	ICP_Time_20	ICP_time_30	ICP_Mean>15	ICP_Mean>20
pre6H_pct_ICPgt20	0.000	0.000	0.000	0.000
pre6H_pct_ICPgt30	0.000	0.000	0.000	0.000
pre6H_PTD_ICPgt20	0.000	0.000	0.000	0.000
pre6H1Q_ICP	0.000	0.000	0.000	0.000
pre6H2Q_ICP	0.000	0.000	0.000	0.076
pre6H3Q_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_ICP	0.000	0.000	0.000	0.000
pre6H_Min_ICP	0.001	0.699	0.000	0.561
pre6H_Max_ICP	0.000	0.000	0.000	0.000
pre6H_Mean_CPP	0.003	0.000	0.000	0.001
pre6H_Min_CPP	0.010	0.001	0.001	0.001
pre6H_Max_CPP	0.013	0.000	0.000	0.053
pre6H_Mean_MAP	0.409	0.709	0.562	0.348
pre6H_Min_MAP	0.251	0.764	0.451	0.770
pre6H_Max_MAP	0.325	0.315	0.167	0.078
pre6H_stdSBP	0.230	0.386	0.299	0.651
pre6H_stdDBP	0.939	0.614	0.230	0.167
pre6H_stdHR	0.397	0.790	0.650	0.650
pre6H_Mean_HR	0.458	0.351	0.206	0.650
pre6H_Mean_SBP	0.930	0.577	0.890	0.414
pre6H_Mean_DBP	0.083	0.656	0.698	0.393
IFN-G	0.730	0.709	0.629	0.965
TNF-A	0.261	0.690	0.769	0.862
IL-2	0.345	0.016	0.317	0.939
IL-4	0.944	0.743	0.585	0.791
IL-10	0.589	0.384	0.307	0.105
IL-1B	0.636	0.669	0.164	0.080
IL-6	0.210	0.028	0.005	0.102
IL-8	0.624	0.084	0.035	0.013
GMCSF	0.467	0.231	0.010	0.011
IL12P70	0.762	0.330	0.773	0.810
IL13	0.347	0.961	0.434	0.406
IL5	0.277	0.058	0.191	0.092
IL7	0.825	0.434	0.377	0.094
NSE	0.777	0.585	0.293	0.697
S100B	0.399	0.838	0.970	0.743

For biomarkers, the principal component analysis (PCA) was used to transform the raw data into a set of linear combinations (components) such that each component has the largest variance, which uncorrelated to other components. PCA summaries the correlations among a set of variables with a smaller set of linear combinations. Table 6 collects the loadings of biomarker variables for each principal component. Each column sums up to 1, with each linear coefficient for the combination of normalized value of individual variables. Similarly, Table 7 shows the loadings of medication variables for each principal component.

Table 6. Loadings of Biomarker Variables for Each Principal Component

Biomarker	BMPC1	BMPC2	BMPC3	BMPC4	BMPC5	BMPC6	BMPC7	BMPC8	BMPC9	BMPC10
IFN-G	0.01	0.02	0.01	0.10	0.07	0.00	0.34	0.13	0.04	0.02
TNF-A	0.04	0.08	0.10	0.06	0.15	0.10	0.12	0.08	0.04	0.01
IL-2	0.00	0.08	0.04	0.10	0.12	0.15	0.09	0.20	0.03	0.04
IL-4	0.07	0.14	0.08	0.06	0.01	0.01	0.02	0.01	0.01	0.04
IL-10	0.10	0.11	0.07	0.01	0.07	0.05	0.00	0.04	0.09	0.05
IL-1B	0.10	0.09	0.03	0.14	0.00	0.06	0.09	0.02	0.09	0.05
IL-6	0.13	0.06	0.03	0.08	0.05	0.04	0.04	0.02	0.02	0.09
IL-8	0.11	0.08	0.03	0.04	0.05	0.04	0.01	0.00	0.13	0.04
GMCSF	0.12	0.04	0.16	0.00	0.00	0.04	0.01	0.01	0.01	0.04
IL12P70	0.06	0.04	0.16	0.13	0.02	0.00	0.07	0.04	0.09	0.06
IL13	0.01	0.04	0.07	0.09	0.08	0.29	0.04	0.08	0.03	0.08
IL5	0.02	0.04	0.03	0.01	0.15	0.14	0.05	0.30	0.06	0.02
IL7	0.08	0.08	0.06	0.08	0.06	0.03	0.02	0.03	0.12	0.24
NSE	0.10	0.03	0.10	0.03	0.10	0.04	0.02	0.06	0.10	0.02
S100B	0.07	0.09	0.03	0.08	0.07	0.01	0.05	0.00	0.13	0.20

Table 7. Loadings of Medication Variables for Each Principal Component

Component	Medication	Med PC1	Med PC2	Med PC3	Med PC4	Med PC5	Med PC6	Med PC7	Med PC8	Med PC9	Med PC10
Sedation	Precedex	0.06	0.28	0.08	0.34	0.08	0.02	0.00	0.02	0.04	0.03
	Propofol	0.30	0.04	0.04	0.03	0.08	0.05	0.05	0.02	0.18	0.03
Pain	Fentanyl	0.25	0.13	0.12	0.02	0.02	0.02	0.02	0.01	0.06	0.14
Hypertonic	NaCl3%	0.03	0.01	0.03	0.02	0.01	0.01	0.01	0.00	0.05	0.03
	NaCl7.5%	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00
Isotonic	Plyte	0.04	0.04	0.14	0.10	0.14	0.08	0.05	0.06	0.22	0.28
	PlyteBolus	0.00	0.00	0.01	0.01	0.02	0.01	0.02	0.04	0.07	0.16
	NaCl0.9%	0.14	0.09	0.19	0.06	0.21	0.01	0.04	0.04	0.20	0.07
	NaCl0.9%Bolus	0.01	0.00	0.02	0.01	0.00	0.01	0.00	0.00	0.03	0.01
	LR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.06
Vasopressor	Mannitol	0.04	0.37	0.19	0.21	0.02	0.00	0.00	0.00	0.00	0.01
	Epinephrine	0.01	0.00	0.01	0.01	0.02	0.75	0.01	0.02	0.03	0.01
	Norepinephrine	0.12	0.02	0.15	0.16	0.34	0.01	0.00	0.04	0.05	0.10
	Phenylephrine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04
	Vasopressin	0.00	0.01	0.02	0.01	0.04	0.02	0.78	0.74	0.04	0.02

For the ICP and CPP calculated variables, the data is 90% available. Features related to SBP and HR had 100% availability. For biomarkers, their availability is 88.2%, and the medication PCA features are 98% available.

4.2 The Mixed Models

The observed data had temporal structure, namely, each patient was repeatedly observed for blood samples, medications, physiologic status, and the corresponding outcomes. The observations within a patient were not completely independent. To take such dependency into consideration, we used a mixed model to handle the individual-specific random effect. In the mixed models, a variable is called fixed effect, if its levels represent all possible levels. If a factor effect is random, if it only represents a limited sample of all possible levels [20]. The variables mentioned in the last section were used as the fixed effect variables. The individual subject ID was used as the variable for the patient-specific random effect. The fixed effect models used logistic regression, with feature selection based on the Wald test of each coefficient's statistical significance in the model.

The mixed models take the dependency within each patient into consideration, based on the assumption that such dependency cannot be omitted. Compared with the vanilla logistic regression model, the mixed models explicitly model individual changes, which is good for fitting models with repeated measurements. Also, the mixed models could provide a flexible modeling framework for multi-center studies, to address random effects from different study centers. However, we still noticed the limitation from the mixed model that it uses the logistic regression and suffer from missing value situation.

4.3 Prediction Models with the Boosting Method

With variables calculated from data in the past 6-hour of each blood sample, we build prediction models to predict outcomes (ICP elevation or CPP decreasing) in the next 6 hours. There are dual objectives when creating those models. First, models should have good accuracy in predicting the future outcomes. Second, models could help to evaluate the variables' contribution to the prediction. As there are many missing values in this dataset, some classification techniques such as logistic regression cannot be applied unless all the cases with missing values are removed, which is not ideal because that would result in even fewer data points for model training. In this situation, decision-tree-based techniques are desired because they are able to handle missing values. Therefore, a decision-tree-based model, called boosting tree, will be used in this study.

Boosting is a machine learning technique used for regression and classification problems. It produces a prediction model in the form of an ensemble of weak prediction models, typical decision trees. That is to say, it usually consists of iterative learning of weak rules. If there is any prediction error caused by preceding weak learners, then it pays higher attention to those observations in the next round of training. Finally, it combines the outputs from weak learners and creates a single strong learner.

The boosting tree method uses an ensemble of decision trees as base models. It inherits the merits of decision tree. First, each base model uses makes no assumptions of the training data or prediction residuals, which is very unlike logistic regression. Second, the boosting tree models are still white-box models. Third, the boosting tree model can handle variables with missing values, which could enhance the models' practical use in real situation with partially observed variables. The boosting tree method also avoids some disadvantages from the decision tree method. First, through a weighting factor, the correction that new decision tree could add to the model is regularized to prevent overfitting the data. Such weighting factor is called the learning rate, which often has values less than 1. Second, boosting tree often uses decision trees with shallow depth (weak learners), so that each tree model only learns a small part of the data. In this way, the models may have better generalization and are robust when they are applied to unseen new data.

The boosting tree provides a way to evaluate the importance of each variable in the prediction model. It calculates the relative number of observations related to each feature. It also calculates the percentage of each feature occurring in the trees. For each single decision tree, the importance score is calculated as the amount that each attribute split point improves the performance measure, weighted by the number of observations the node is responsible for. The performance measure may be the purity (Gini index) used to select the split points or another more specific error function. A variable's overall importance is calculated by averaging its importance scores across all of the decision trees within the model. A higher value of importance score of a variable means it is more important in contributing to the prediction in the model.

To evaluate the models' prediction performance, especially on unseen new data, we used the cross-validation. Considering that each subject had repeated observation (up to 13 times), we validate the models at the subject level, instead of at the individual observation level. This validation scheme better simulates the model's practical application scenario, namely using it for predicting an entirely new subject's future outcomes. Given the collected data, we use ~90% of subjects for training, use ~10% (5 patients) for testing by random sampling from the dataset.

Such process was repeated for 100 times. The performance measure, area under the receiver operating curves (AUROCs) is then averaged among all the tests.

4.4 Prediction of Mortality and Neuroworsening

In previous sections, 'fit-to-fly' was considered as ICP or CPP having no long episode of abnormal values. In this section, we consider two other outcomes that may be used in making decision for transporting a patient, one is in-hospital mortality and one is neurologic deterioration (neuroworsening).

Invasive methods like ICP monitoring and noninvasive methods like regular interval CT scans have been developed to monitor these patients and to quickly identify changes. The role of the initial brain CT scan and of repeat brain CTs is well established. Serial CT scans in head trauma are obtained for early capture of neurologic worsening which can lead to early medical and surgical interventions even before the clinical symptoms manifest [21]. Baseline CT head (CT-B) performed at the time of admission and interval CT scans at approximately 6 +/- 3 hours (CT1) and 24 +/- 6 hours (CT2) post admission were reviewed. Marshall Classification of CT-B was used to assess the severity of brain injury at the time of admission. The Marshall scoring was done by a trained physician. Interval CT scans were compared to CT-B using 4 variables – contusion, ischemia, compression of basal cisterns and midline shift. The comparison resulted in 2 possible outcomes on the interval CT –worsened or not worsened. The outcomes were cross validated by verifying the radiology reports read by attending radiologists. In this way, two evaluations for neurological worsening (NW) were obtained at the 6 hours and 24 hours after ICU admission.

4.5 ICP Estimation and Prediction

Above experiments show that invasive VS derived variable could have better prediction performance than other variables in estimating future ICP status. We may ask can we estimate the near future ICP values using available VS. If such estimation is possible and satisfy some error criteria, the predicted ICP could be used to push the prediction horizon further. Assume that continuous VS, such as ICP, SBP, HR, etc. are collected. How accurate can we estimate the ICP values for the next 5 minutes to 2 hours?

Recently, nearest neighbor regression (NNR) has been applied for predicting ICP for patients with VS -including HR, SBP, shock index (SI), mean arterial pressure (MAP), pulse pressure (PP), and ICP itself [42]. On this basis, patients' status has been represented with HR, SBP, SI, MAP, PP, and ICP in the current and past 5, 10, 15, and 20 minutes. For each subject, state of the patient is compared with all previous observations, and *k* nearest states are passed to the regression model for estimating the ICP in next 5 minutes [22].

Although using NNR for estimating ICP has initially demonstrated promising results, performance of the predictive model is ebbed during the time. In some sense, the more observation a system collects, the higher response time and lower performance the system may face. To address all drawbacks in employing NNR for predicting ICP for patients with severe TBI, a novel approximate NNR has been designed and developed in this study. To this end, similarity preserving hashing techniques has been adapted to reduce the response time of NNR from linear search to constant time in this novel representation of VS.

Owing to the nature of NNR, every single system state is compared with all previous observations to recognize nearest system states (linear search). Hence, response time for NNR based predictor is increased gradually due to adding new observations into the repository. This issue is tied with aggressive memory utilization of NNR. More precisely, not only velocity of recording VS and storing patients' states is high enough to sort out the problem as big data application, but fetching items from the repository also gets slower due to memory management concerns.

Locality sensitive hashing (LSH) is a novel approach for compressing and representing large feature spaces through which, cost of finding similar observations (both time and memory) is substantially subsided -compare to standard nearest neighbor search techniques [23]. In this trend, a set of randomly chosen hash functions are applied on observation vectors in such a way that similarity of original instances in feature space is preserved in hash space. Generally, similarity of instances is defined using a distance metric (e.g. Euclidean distance for ICP prediction with NNR), and each family of hash functions preserve the similarity of specific distance metric.

From feature engineering perspective, each hash function hashes the observation vector to bucket numbers statistically independent from other hash functions. And, in contrast to traditional hashing techniques in cryptography that try to minimize the probability of collision for identical items, LSH indexes similar items in same buckets. In this sense, the more similar system states, the higher probability of hashing them to same bucket. Consequently, LSH retrieves potential nearest neighbors in constant time -only system states in same buckets are compared to identify nearest neighbors, rather than checking all observations in the repository. It is also worth noting that FP and FN rates in preserving the similarity of system states is adjustable through amplifying the locality sensitive hash functions. Finally, LSH is significantly less sensitive to missing data. Locality sensitive hashing applies simple random projections that are independent from the data. Therefore, LSH requires long binary codes to achieve satisfactory performance [24]. As illustration, using 400 randomly chosen hash function will demonstrate 95% similarity preserving accuracy; however, to enhance the similarity preserving accuracy to 98%, more than 900 statistically independent hash functions are needed. To address this issue, double-bit quantization for hashing (DBQ) has been adapted in this study to train hash functions to generate substantially short similarity preserving hash codes [25]. To this end, principle PCA initially transfers data from feature space into significantly lower dimension in principle component space. Afterwards, two bits are used to quantize values of each principle component according to two adaptive thresholds that are adjusted for each dimension separately.

Consequently, in contrast to NNR approach for predicting ICP that similarity of system states had been defined via Euclidean distance, in this study hamming distance between hash codes have been employed to measure how close two input vectors are. Apparently, not only DBQ requires substantially less memory compare to NNR approach, but measuring the similarity of VS pairs require significantly less time than NNR and LSH as well.

5.0 RESULTS

5.1 Results for the Mixed Models

In the experiments, we used various combinations of age, Marshall Score, SVS, ICM, CYT and medication variables. For the 50 subjects with ICP measurements, there were total 488

times of observation. Due to missing values among the variables, the mixed models removed the observations with missing values. Therefore, each model may have used different number of observations (N). From Tables 8-11, we can observe that the mixed models with invasive VS ICM were in general with higher AUROC. For example, in predicting ICP>20mmHg for >30 min, the mixed model MM14, which used only ICM variables, had AUROC 0.89 (95% confidence interval 0.86-0.93). MM1 added SVS to MM14 and had AUROC 0.90 (95%CI 0.87-0.93), which is not significantly different from MM14's AUROC. Similarly, by adding CYT or medication variables, there was not significant improvement. When the ICM variables were absent, models that used medication information, such as MM16 had AUROC 0.70 (95%CI 0.65-0.76).

For models that had no ICM variables, they could benefit more from using age or Marshall Score information. While for models that used ICM variables, their models' AUROCs were not influenced by extra information from age or Marshall Score.

Table 8. Mixed Models and their AUROCs in Predicting ICP>20mmHg for >30min

Name	N	AUROC	95% Wald CL		Age	Marshall Score	SVS	ICM	CYT	Medication
MM1	390	0.90	0.87	0.93			X	X		
MM2	390	0.90	0.87	0.93			X	X	IL10	
MM3	433	0.75	0.70	0.80	X	X			IFN, IL5, IL7	
MM4	433	0.52	0.46	0.57					IFN, IL5, IL7	
MM5	475	0.72	0.67	0.77	X	X	X			
MM6	475	0.54	0.50	0.59			X			
MM7	390	0.90	0.87	0.93				X	IL5, IL10	
MM8	390	0.90	0.87	0.93				X	IL5, IL10	
MM9	390	0.90	0.87	0.93			X	X		
MM10	390	0.90	0.87	0.93			X	X		
MM11	422	0.77	0.72	0.82	X	X	X		IFN, IL5, IL7	
MM12	420	0.57	0.51	0.63			X		TFN, IL5	
MM13	440	0.89	0.86	0.93	o	o		X		
MM14	440	0.89	0.86	0.93				X		
MM15	481	0.77	0.72	0.81	X	X				MedPC2,3,5
MM16	481	0.70	0.65	0.76						MedPC2,3,5
MM17	414	0.82	0.78	0.86	X	X	X		IL5, IL12	MedPC2,3,5
MM18	414	0.74	0.69	0.80			X		IL5, IL12	MedPC2,3,5

Note: Those models selected variables among age, Marshall Score, SVS, ICM, CYT, and medication. ('o' means the variables were considered, but not selected by the algorithm).

Table 9. Mixed Models and their AUROCs in Predicting ICP>30mmHg for >15min

Name	N	AUROC	95% Wald CL		Age	Marshall Score	SVS	ICM	CYT	Medication
MM1	391	0.91	0.88	0.95		X	X	X	IFN, TNF, IL2,IL7	
MM2	289	0.88	0.83	0.92			X	X	IL2	
MM3	430	0.75	0.68	0.82	X	X			IFN,TNF, IL2	
MM4	431	0.52	0.44	0.59					TNF	
MM5	475	0.75	0.68	0.82	X	X	X			
MM6	475	0.60	0.52	0.68			X			
MM7	387	0.91	0.88	0.95		X		X	IFN, TNF, IL2,IL7	
MM8	387	0.88	0.84	0.93				X	TNF, IL2, IL8	
MM9	440	0.90	0.86	0.94		X	X	X		
MM10	440	0.88	0.84	0.93			X	X		
MM11	419	0.82	0.75	0.88	X	X	X		IFN, TNF, IL2,IL7	
MM12	420	0.62	0.54	0.71			X		TNF, IL12	
MM13	440	0.89	0.85	0.93		X		X		
MM14	440	0.88	0.84	0.93				X		
MM15	481	0.69	0.61	0.77						MedPC2,3,5
MM16	473	0.79	0.73	0.85	X	X				MedPC2,3,5
MM17	411	0.86	0.80	0.92	X	X	X		IFN, TNF, IL2, IL7, IL12	MedPC2,3,5
MM18	412	0.74	0.66	0.83			X		TNF, IL8, IL12	MedPC2,3,5,6

Note: Those models selected variables among age, Marshall Score, SVS, ICM, CYT, and medication.

Table 10. Mixed Models and their AUROCs in Predicting CPP<50mmHg for >15min

Name	N	AUROC	95% Wald CL		Age	Marshall Score	SVS	ICM	CYT	Medication
MM1	386	0.88	0.83	0.92			X	X	IL2	
MM2	386	0.88	0.83	0.92			X	X	IL2	
MM3	424	0.77	0.70	0.84	X	X			TNF	
MM4	424	0.63	0.55	0.71					TNF, IL8	
MM5	469	0.83	0.77	0.88	X	X	X			
MM6	469	0.74	0.68	0.81			X			
MM7	386	0.86	0.81	0.91		X		X	IL2	
MM8	386	0.84	0.79	0.90				X	IL2	
MM9	437	0.88	0.83	0.92			X	X		
MM10	437	0.88	0.83	0.92			X	X		
MM11	414	0.81	0.75	0.88		X	X		TNF	
MM12	416	0.74	0.67	0.81			X		IL10	
MM13	437	0.87	0.82	0.92		X		X		
MM14	437	0.85	0.79	0.90				X		
MM15	471	0.80	0.74	0.87	X	X				MedPC5,6
MM16	471	0.54	0.46	0.63						MedPC3,4,5,6
MM17	461	0.82	0.76	0.87	X	X	X			MedPC3,5
MM18	408	0.75	0.69	0.82			X		IL10	MedPC5,6

Note: Those models selected variables among age, Marshall Score, SVS, ICM, CYT, and medication.

Table 11. Mixed Models and their AUROCs in Predicting CPP<60mmHg for >30min

Name	N	ROC	95% Wald CL		Age	Marshall Score	SVS	ICM	IFN	Medication
MM1	387	0.86	0.82	0.89		X	X	X	NSE	
MM2	387	0.85	0.82	0.89			X	X	IL6, IL8	
MM3	426	0.71	0.66	0.76		X			IL8	
MM4	426	0.62	0.58	0.67					IL8	
MM5	469	0.77	0.73	0.82		X	X			
MM6	469	0.74	0.69	0.79			X			
MM7	387	0.85	0.81	0.89		X		X	NSE	
MM8	387	0.83	0.78	0.87				X	IL6, IL7	
MM9	437	0.87	0.83	0.90		X	X	X		
MM10	437	0.85	0.82	0.89			X	X		
MM11	416	0.77	0.73	0.82		X	X		NSE	
MM12	416	0.74	0.69	0.79			X		IL8, NSE	
MM13	437	0.86	0.83	0.90		X		X		
MM14	437	0.84	0.80	0.88				X		
MM15	471	0.76	0.71	0.81	X	X				MedPC3,4,6,7
MM16	471	0.63	0.58	0.69						MedPC2,3,6
MM17	408	0.80	0.76	0.85		X	X		IL10, NSE	MedPC2,3
MM18	408	0.78	0.73	0.83			X		IL7, IL8, NSE	MedPC2,3

Note: Those models selected variables among age, Marshall Score, SVS, ICM, CYT, and medication.

5.2 Results for the Boosting Tree Models

Figure 2 summaries the training (blue dots) and testing (red dots) for the four outcomes. The AUROCs on training datasets show how well the models fit the data. The AUROCs on testing datasets show the expected performance of the models on unseen new data. Models with invasive VS (M6 and M7) had higher AUROCs in both training and testing datasets. Models that use non-invasive VS, biomarkers and medication (M5) had AUROC = 0.76 in predicting

$CPP_{<60mmHg}$ > 30min. Compared with other models, the biomarker models did not show significantly higher AUROCs in predicting the four outcomes.

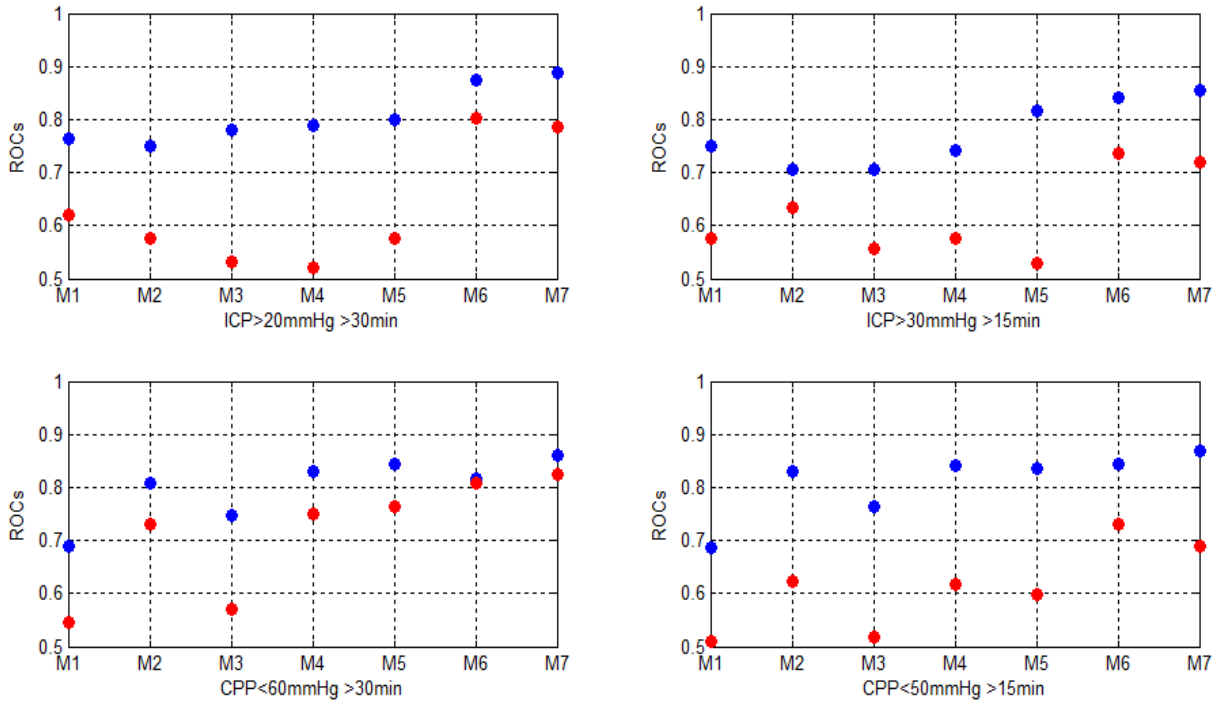


Figure 2. Averaged AUROCs for training (blue) and testing (red) for models using different groups of variables. M1: medication variables; M2: non-invasive VS; M3: biomarker variables; M4: non-invasive VS + biomarkers; M5: non-invasive VS + biomarkers + medication; M6: invasive VS; M7: all groups.

It is important to understand how each variable's contribute to the prediction. As aforementioned, the boosting tree models evaluate each variable and rank its importance with a score. A higher score of a variable indicates that it is more important in contributing to the prediction in the model. Use the prediction for the outcome ICP<30mmHg for >30min as an example. The model built on non-invasive VS had variable importance score ranking shown in Figure 3. Variables that describe the center and extreme values of the data were ranked higher than the variables that describe the dispersion of data.

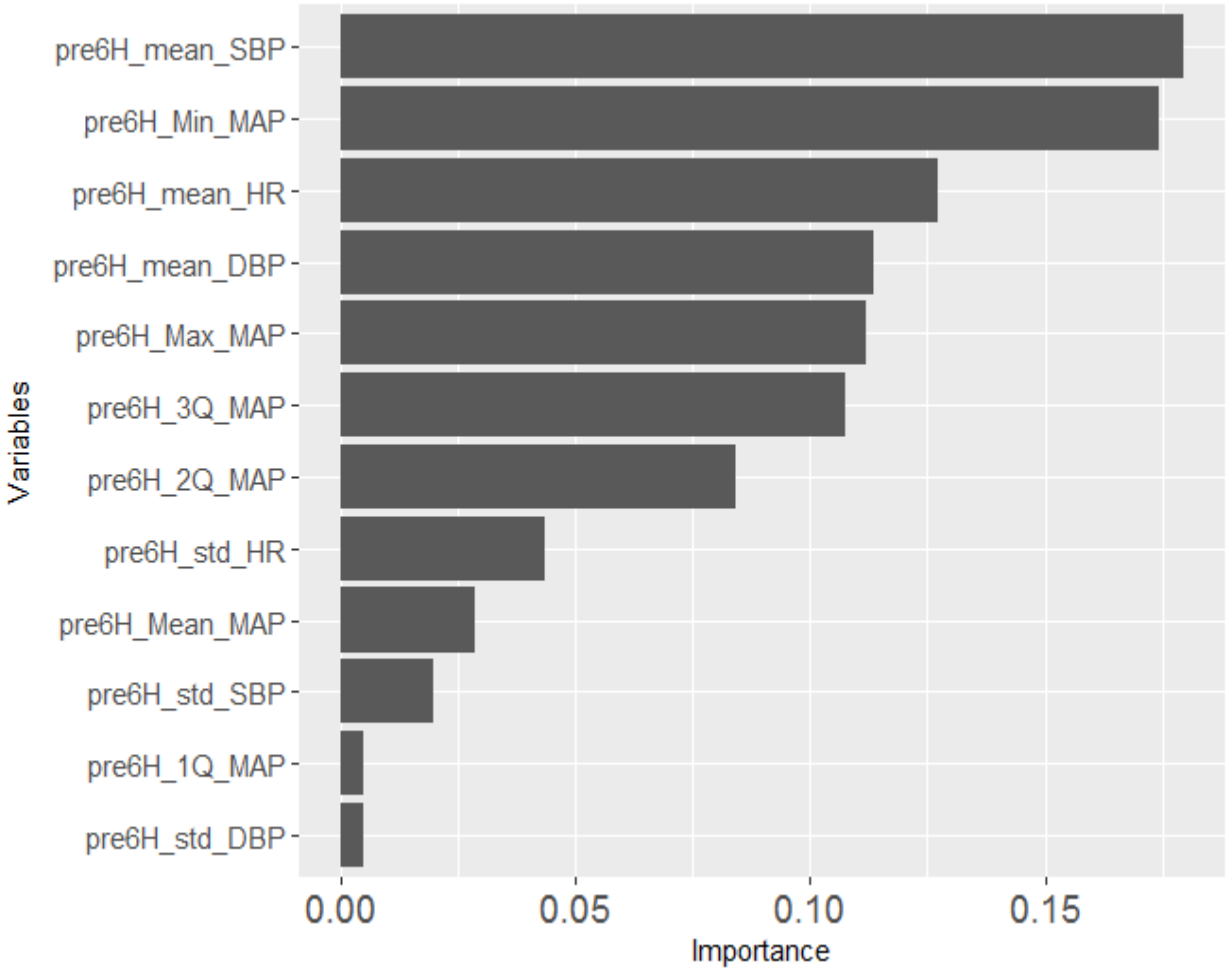


Figure 3. Variable importance ranking for the variable group of non-invasive VS in predicting ICP >30mmHg for > 15min.

For the biomarkers, Figure 4 shows the importance ranking of the raw biomarkers and the principal components together. For the raw biomarkers, IL-8, IL-2, and IL-5 are among the top importance cytokines. For the principal components, BMPC 9 is among the top importance. From Table 6, we can find that IL-8, S100-β, IL-7 are the top three contributors to the BMPC 9. Similarly, Figures 5 and 6 show the importance in models that use only one group of variables from invasive VS or medications.

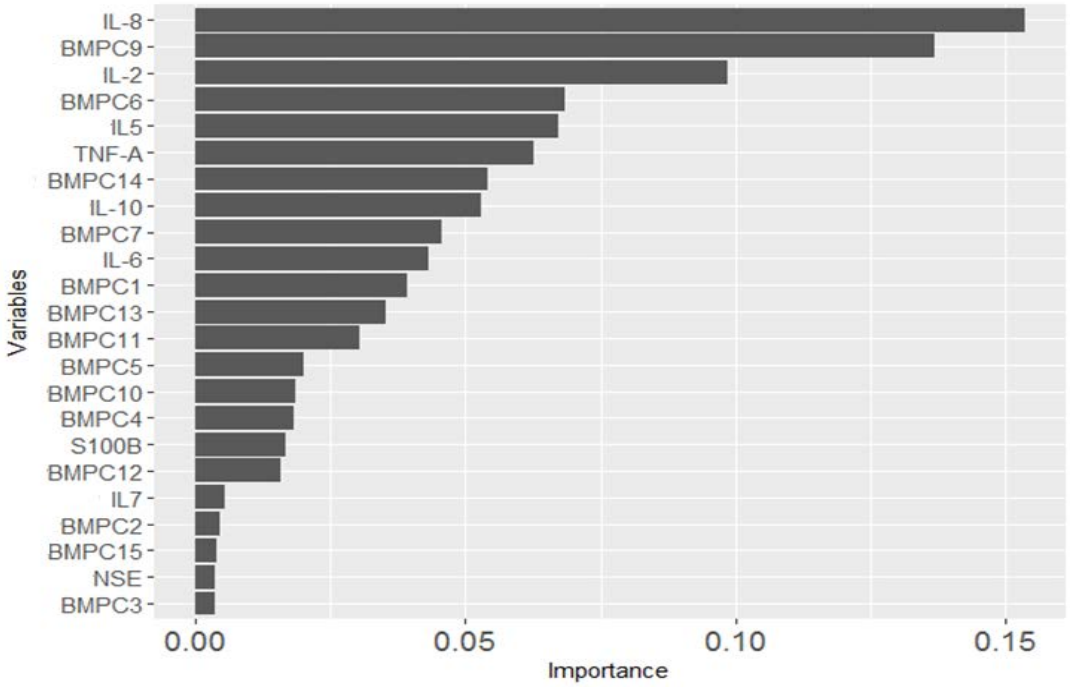


Figure 4. Variable importance ranking for the variable group of biomarkers in predicting ICP >30mmHg for > 15min.

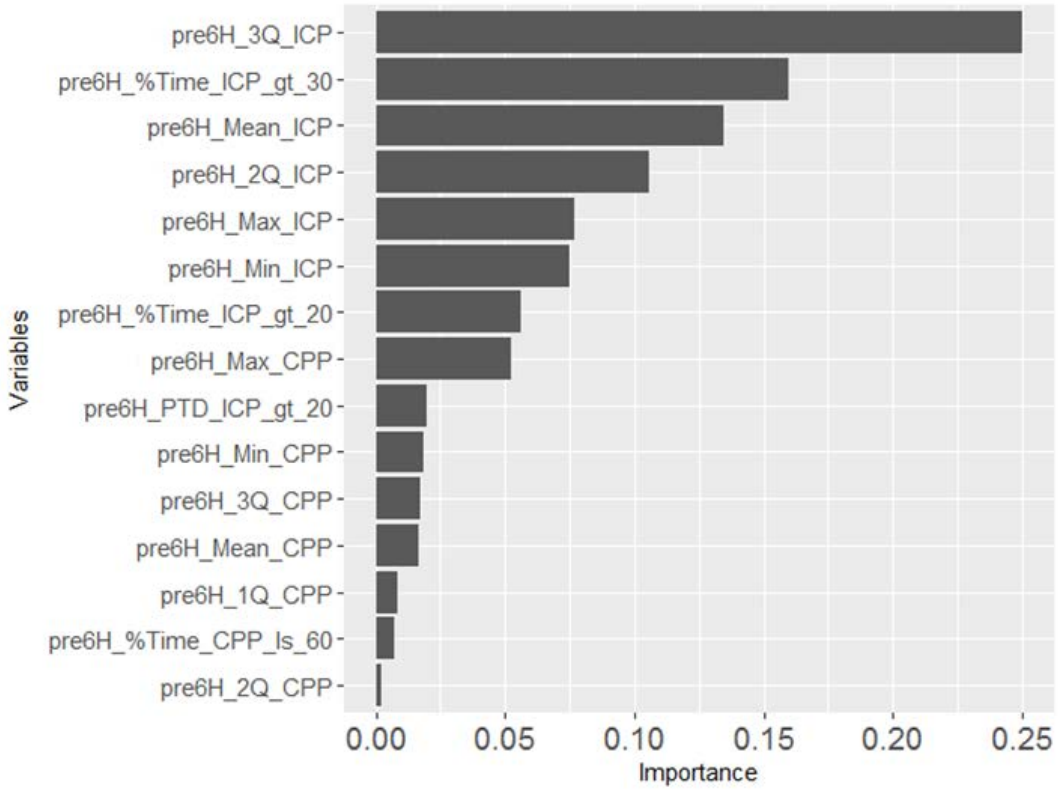


Figure 5. Variable importance ranking for the variable group of invasive VS (ICP) in predicting ICP >30mmHg for > 15min.

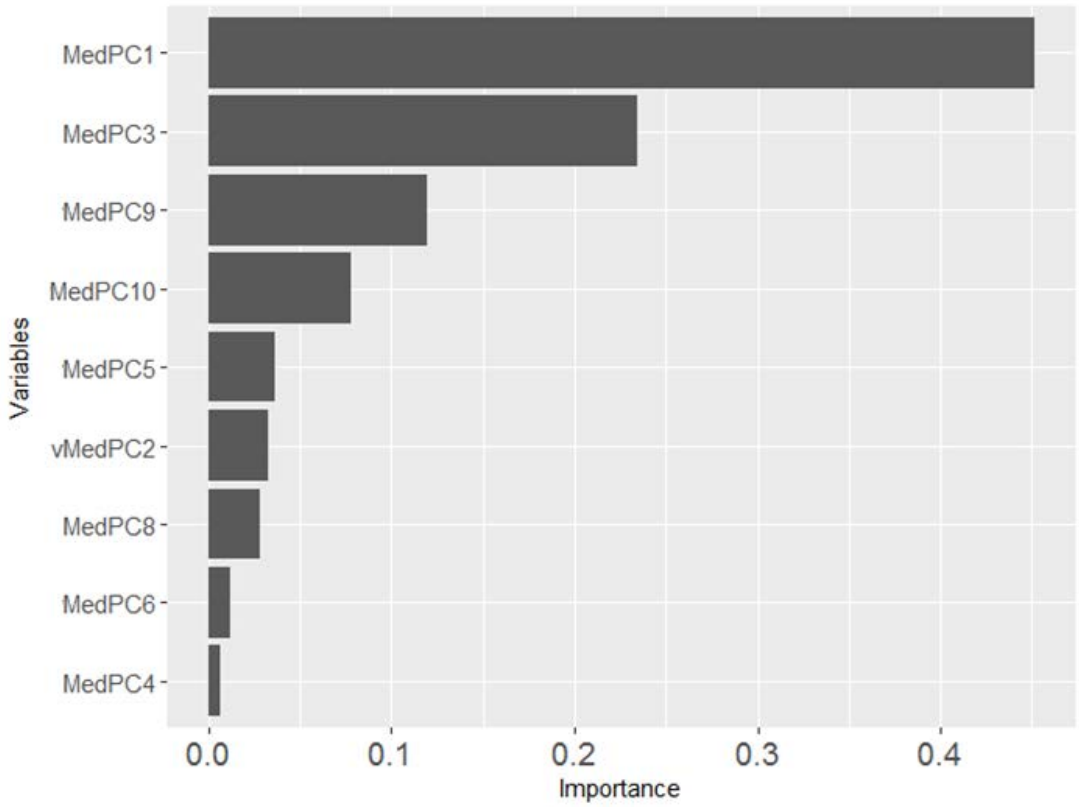


Figure 6. Variable importance ranking for the variable group of medication in predicting ICP >30mmHg for > 15min.

Instead of evaluating each variable group individually, all types of variables were used together and ranked for their contribution to the outcome prediction. Figures 7-10 summarize the ranking for all types of variables in prediction ICP>20mmHg for >30min, ICP>30mmHg for >15min, CPP<60mmHg for >30min, and CPP<50mmHg for >15min. Using the Figure 7 as an example, we can see that invasive VS (ICP) related variables are the top three most important contributors to the ICP elevation prediction. Next to them is a principal component from the biomarker variables, the BMPC 9. As the Table 4 shows, the BMPC 9 is mainly explained by IL-8 (13.03%), S100β (12.70%), and IL-7 (11.96%). A principal component from the medication, MedPC 1 is also ranked on the fifth important variable. From Table 5, we can find that MedPC 1 is mainly explained by Propofol (30.09%), Fentanyl (24.58%), and NaCl0.9% (13.60%), for the purposes of sedation, pain management, and hypertonic.

The top five most important variables contain 3 variable groups. Although the models built entirely from biomarker variables had lower AUROCs than the ones built from invasive VS, this overall ranking still shows that part of biomarker variables are very important and have strong relations to the outcomes. For example, BMPC 4 and BMPC 1 are next to the invasive VS derived variables. They are mainly explained by IL-1B (13.95%), IL-2P70 (13.34%), IL-6 (12.82%), IL-8 (11.31%), and GMCSF (11.73%).

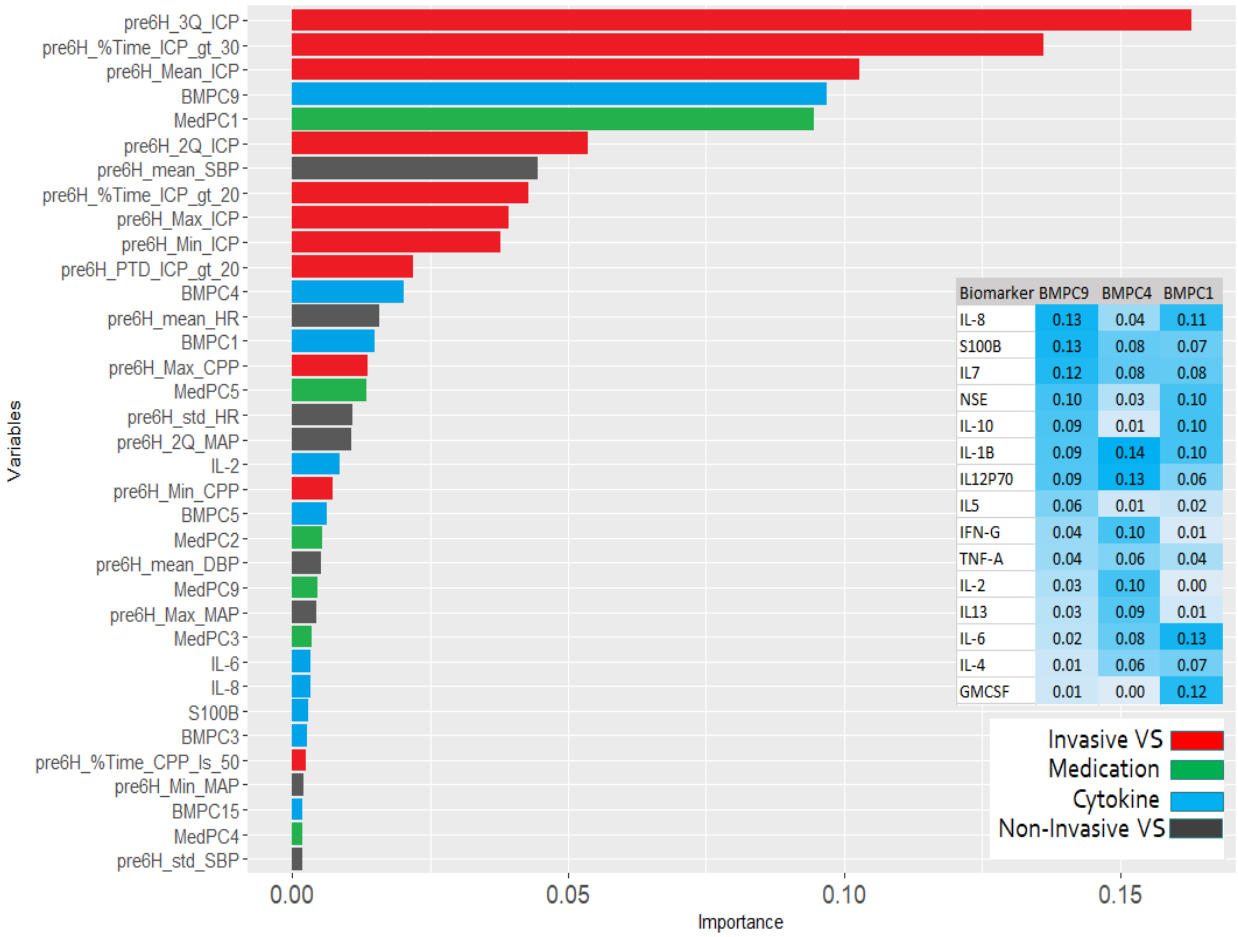


Figure 7. Variable importance ranking for all variable types in predicting ICP >30mmHg for > 15min.

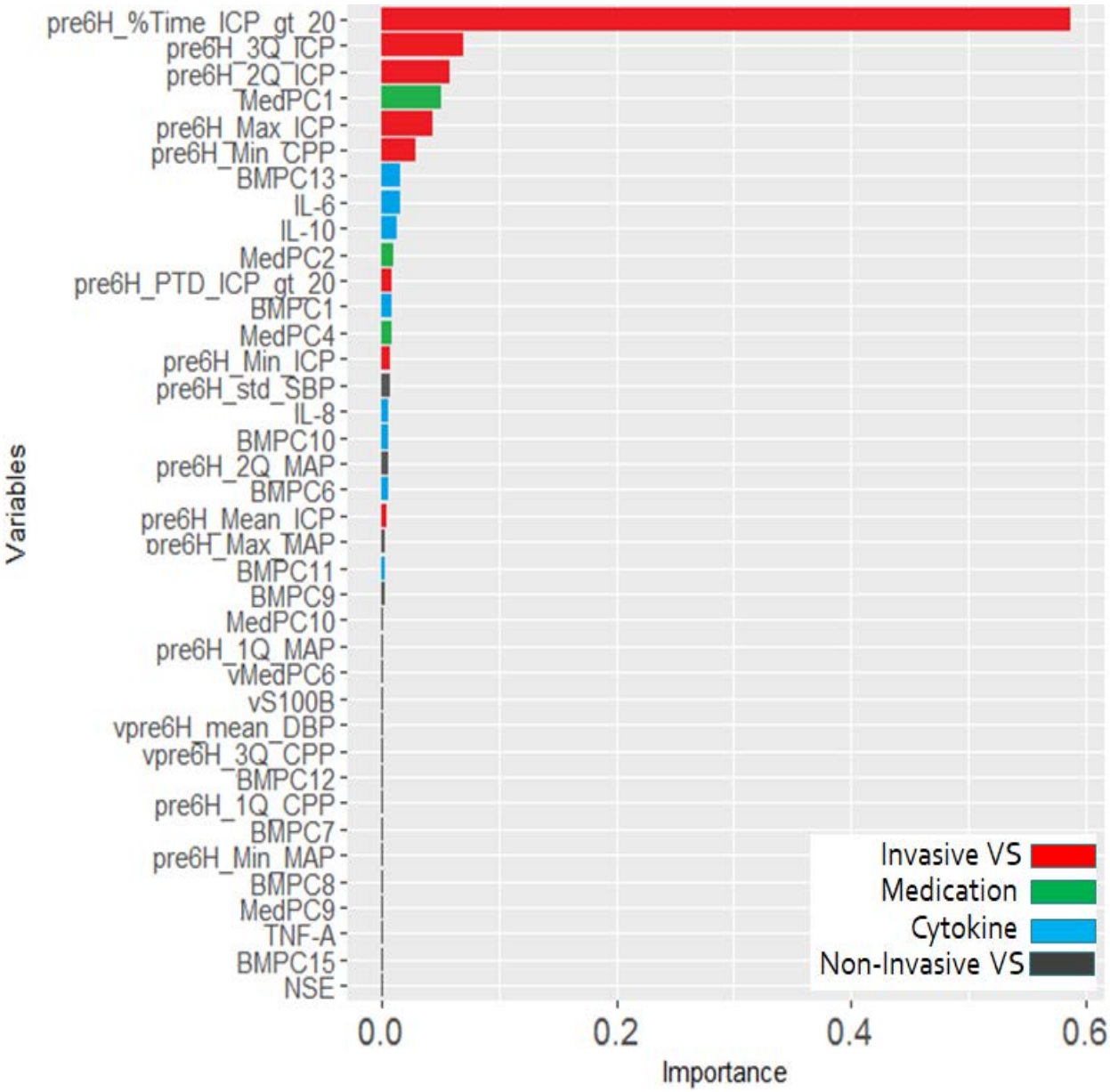


Figure 8. Variable importance ranking for all variable types in predicting ICP >20mmHg for > 30min.

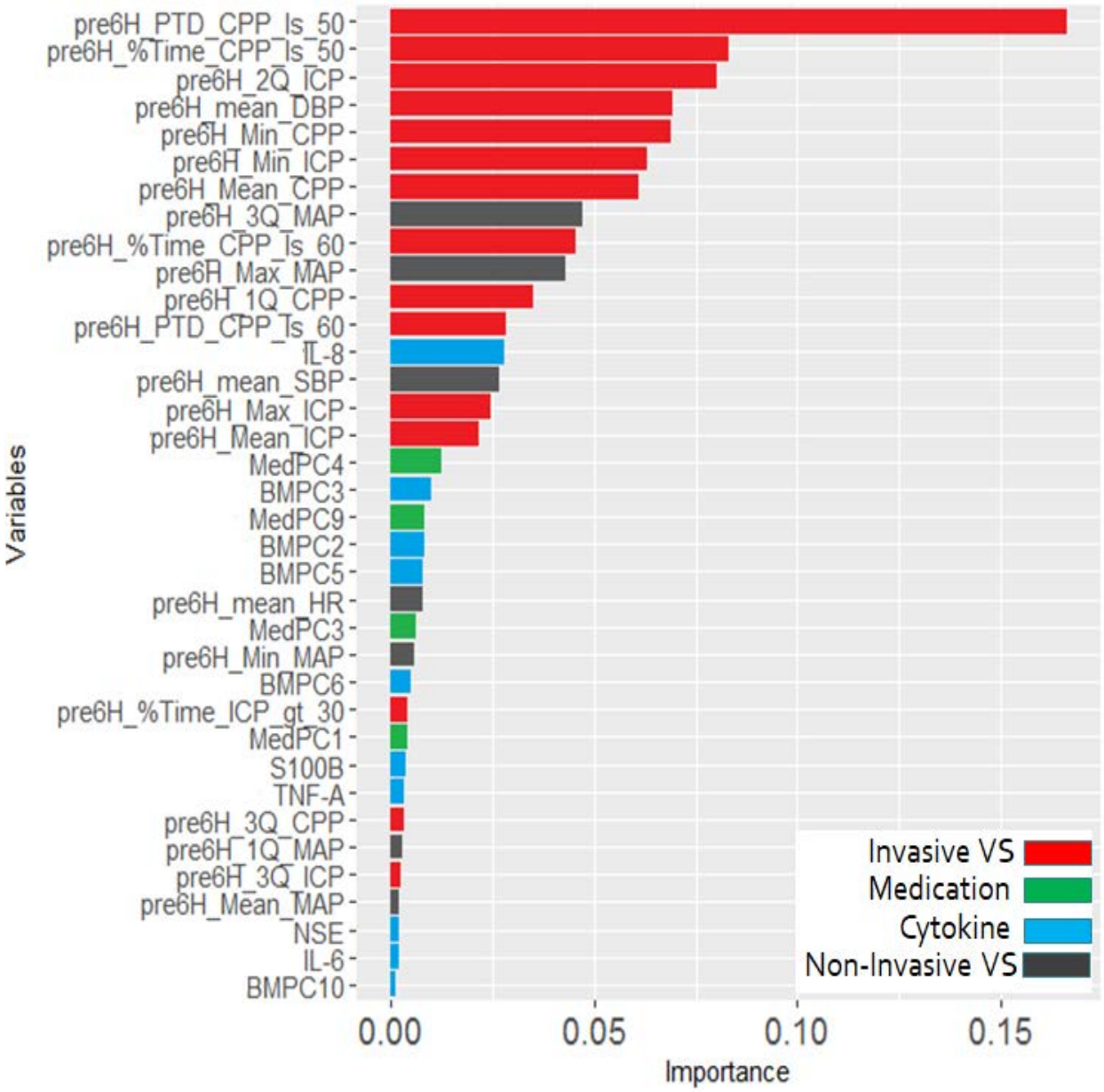


Figure 9. Variable importance ranking for all variable types in predicting CPP < 50mmHg for > 15min.

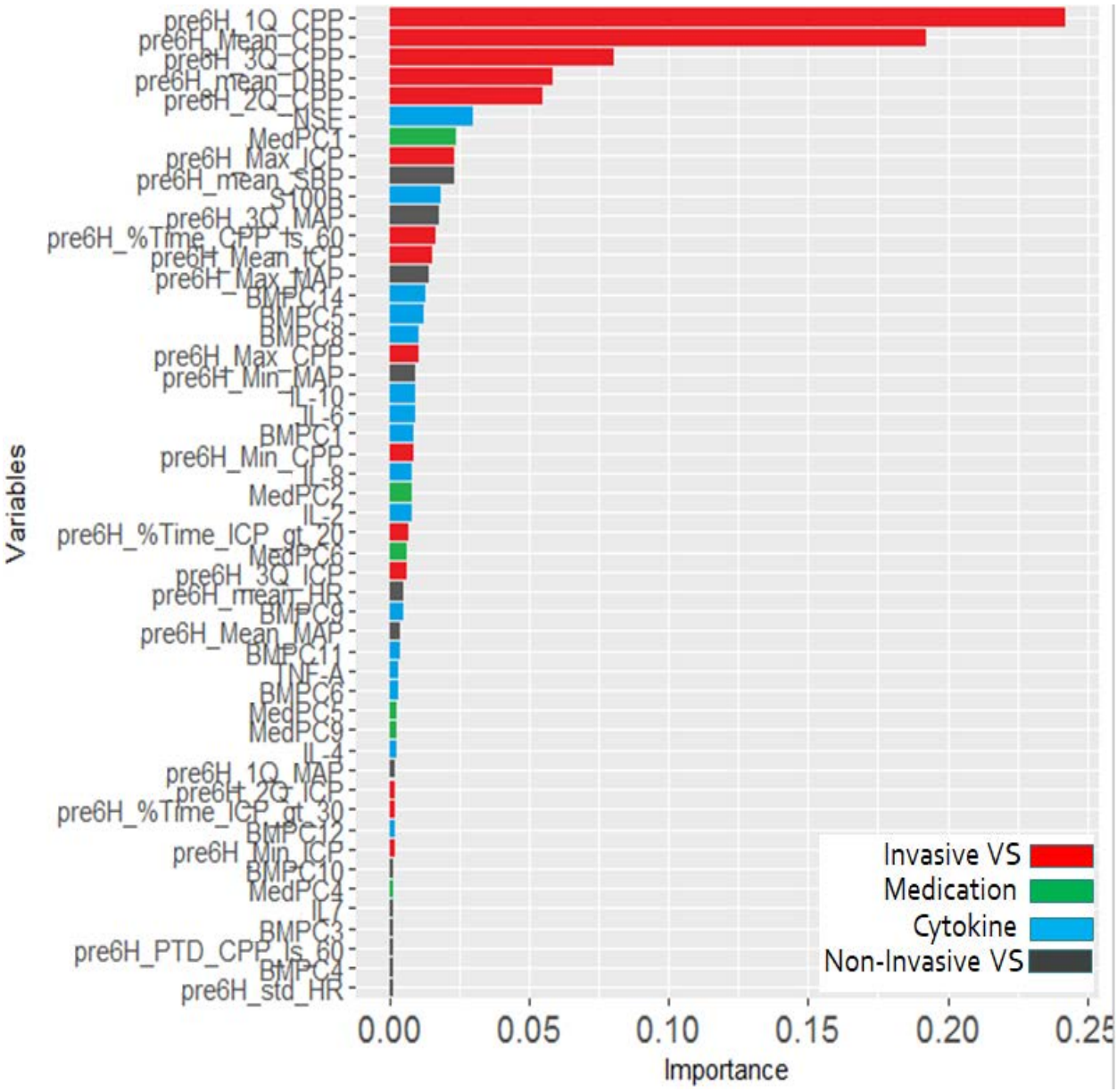


Figure 10. Variable importance ranking for all variable types in predicting CPP < 60mmHg for > 30min.

5.3 Results for Mortality and Neuroworsening Prediction

The 62 enrolled cases and variables in the first 6 hours and the fourth 6 hours were used to predict mortality and the NWs. Tables 12-15 summarize the AUROCs and their 95% CI, as well as the false positive rate (FPR), true positive rate (TPR), false negative rate (FNR) and true negative rate (TNR) for each model in predicting mortality and NWs at 6 and 24 hours. Models with invasive VS ICM still had the highest AUROCs.

Table 12. Model Performances in Predicting In-Hospital Mortality Using the First 6 hours ICU Observations

Models	AUROC	FPR	TPR	FNR	TNR	auc-ci-low	auc-ci-up
SVS	0.64	0.11	0.30	0.70	0.89	0.55	0.73
CYT	0.66	0.38	0.68	0.32	0.62	0.58	0.75
Medication	0.64	0.35	0.59	0.41	0.65	0.56	0.72
SVS+CYT	0.69	0.26	0.60	0.40	0.74	0.61	0.77
SVS+CYT+medication	0.68	0.50	0.87	0.14	0.50	0.61	0.76
SVS+CYT+medication+ICM	0.70	0.42	0.81	0.19	0.58	0.63	0.77

Table 13. Model Performances in Predicting NW at 6 hours Using the First 6 hours ICU Observations

Models	AUROC	FPR	TPR	FNR	TNR	auc-ci-low	auc-ci-up
SVS	0.73	0.45	0.86	0.14	0.55	0.70	0.76
CYT	0.69	0.44	0.78	0.22	0.56	0.64	0.74
Medication	0.55	0.21	0.28	0.72	0.79	0.51	0.60
SVS+CYT	0.76	0.38	0.84	0.16	0.62	0.72	0.81
SVS+CYT+medication	0.68	0.45	0.79	0.21	0.55	0.63	0.74
SVS+CYT+medication+ICM	0.78	0.38	0.89	0.11	0.62	0.74	0.83

Table 14. Model Performances in Predicting In-Hospital Mortality Using the First 24 hours ICU Observations

Models	AUROC	FPR	TPR	FNR	TNR	auc-ci-low	auc-ci-up
SVS	0.76	0.13	0.56	0.45	0.87	0.66	0.86
CYT	0.66	0.06	0.33	0.67	0.94	0.59	0.72
Medication	0.77	0.14	0.63	0.37	0.86	0.73	0.81
SVS+CYT	0.76	0.14	0.60	0.40	0.86	0.69	0.84
SVS+CYT+medication	0.77	0.14	0.64	0.36	0.86	0.73	0.81
SVS+CYT+medication+ICM	0.79	0.17	0.70	0.30	0.83	0.74	0.84

Table 15. Model Performances in Predicting NW at 24 hours Using the Fourth 6 hours ICU Observations

Models	AUROC	FPR	TPR	FNR	TNR	auc-ci-low	auc-ci-up
SVS	0.69	0.36	0.72	0.28	0.64	0.64	0.75
CYT	0.62	0.52	0.76	0.24	0.48	0.58	0.66
Medication	0.76	0.34	0.72	0.28	0.66	0.73	0.80
SVS+CYT	0.68	0.45	0.80	0.20	0.55	0.63	0.74
SVS+CYT+medication	0.79	0.27	0.77	0.23	0.73	0.76	0.82
SVS+CYT+medication+ICM	0.81	0.24	0.78	0.22	0.76	0.78	0.83

5.4 Results for ICP Estimation

From 2013 to 2015, 200 adult patients with Camino intraparenchymal ICP monitoring (>19,000 hours, 19.6% of them ICP≥15 mmHg) were available for this study. Mean age was 42.3 years old (SD=18.7). Patients were mainly male (76.5%), and injured by blunt force (87.9%). VSs were recorded every 2-sec and averaged each one minute. Continuous vital signs (VS) including ICP, heart rate, mean and systolic arterial pressures were collected at a level one trauma center neuro ICU. A patient VS status at a time is represented by a 60-dimension vector which based on the above four VSs in the past 15 minutes (Figure 11).

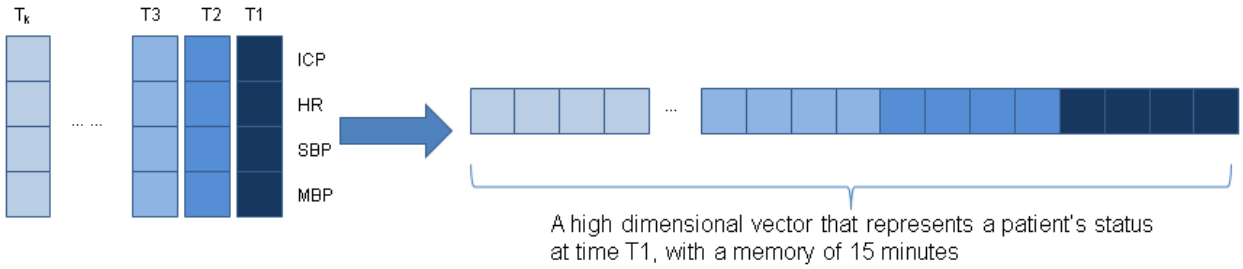


Figure 11. Use past multiple VS measured at k time points (e.g. k = 15 min) to create a vector that represents a patient's status at time T1, with a memory of k minutes.

To predict a patient's near future ICP, hash code for VS status is generated through applying the proposed adaptive similarity preserving method in this study. After assigning the new VS to the buckets, previously assigned VS status vectors to the same buckets are retrieved. Afterwards, standard nearest neighbor search is applied on retrieved VS status vectors to identify nearest experiences. K nearest neighbors' known 'future' trajectories are weighted based on their distance to the new VS status (Figure 12). It is worth noting that the new VS status vector is also added to the corresponding buckets (after observing the actual ICP value) according to its hash signature for future ICP predictions.

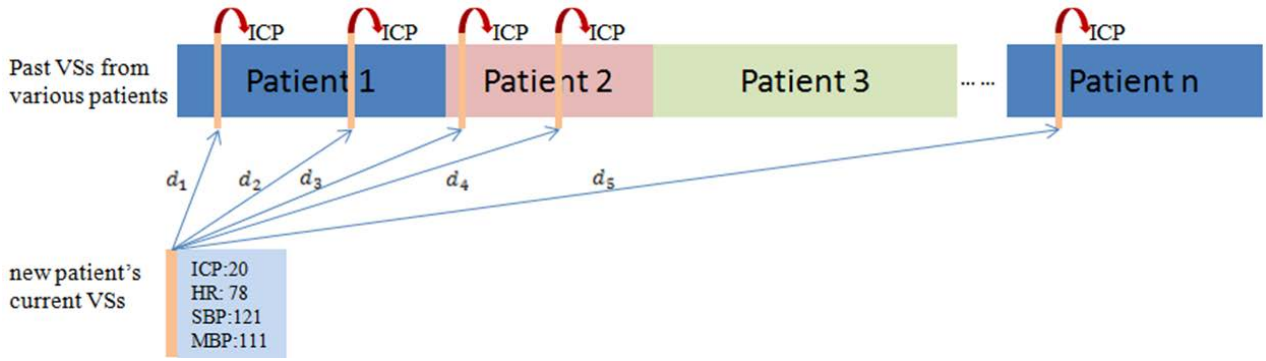


Figure 12. Given a new patient's current VS, search top K similar data point from a dataset consisting past VSs from various patients.

Data are properly normalized, with zero mean and standard deviation. This is to prevent elements with large amplitude dominating the distance. For example, SBP often ranges above 100 mmHg; while ICP often ranges from 0 to 30 mmHg. Without normalization, the distance tends to measure data points with closer SBPs as similar points. Although preprocessing methods are used to normalize raw data as well as removing seasonality of time series in VS status records, surprisingly, similarity preserving hashing technique used in this study also provides implicit raw data preprocessing features. More precisely, similarity-preserving hashing techniques serve same performance functionality with and without intensive preprocessing of raw data.

With identified K nearest data points, the future ICP estimation is done through the weighted average of the top K nearest neighbors. Figures 13-15 demonstrate that the algorithm under- and over-estimated future ICP around 1-hour horizon. However, the estimations were still within acceptable range, since the estimates were the same as the true values being under the 15mmHg warning threshold.

Bland-Altman analysis adjusted for repeated measurement shows that the subsequent 5 minutes prediction and observation have bias of 0.01 mmHg, and 95% limits of agreement ranged from 2.8 to -2.8 mmHg (Figure 16); and bias of 0.1 mmHg, 95% limits of agreements ranged from 7.1 to -6.9 mmHg for the subsequent 2 hours (Figures 17 and 18). This shows that the ICP prediction based on similarity search has good performance in 5-20 minutes future estimation. The estimation performance drops when the prediction horizon goes beyond 1 hour. One possible reason is that when ICP elevation happens, clinicians often would take actions to control it. Given 1 hour or even longer time, many interventions may be provided, which may alter the trajectory of ICP. Hence the prediction of ICP in further horizon becomes more difficult.

With sufficient historical data and efficient searching algorithm, past similar observation could provide reasonable ICP estimations in clinically useful time frames. Further study including the bed side treatment may enhance the prediction power and lead to a real-time bedside ICP trajectory monitor.

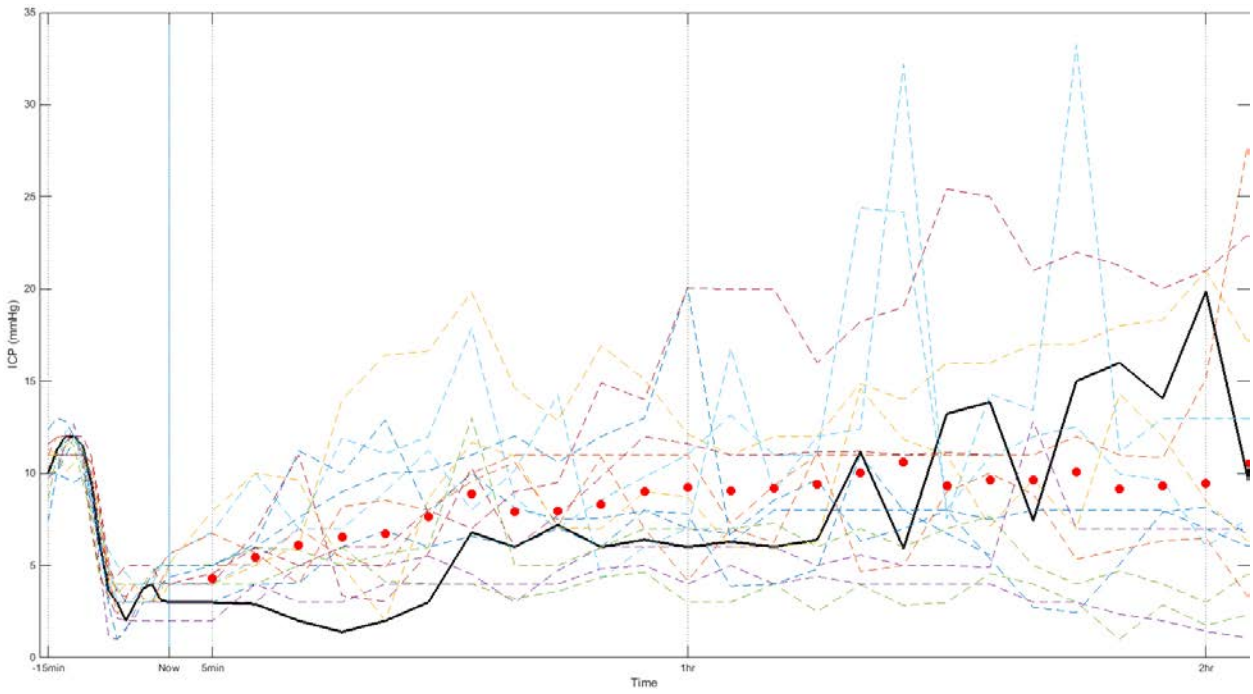


Figure 13. An example of finding similar patients (dash lines) given a new patient past 15 minutes observation (solid line on the left of time 'Now'). Red dots are predictions of future 5 minutes to 2hr ICP. This example shows over-estimated values in the next 1hr, which however are still under 10mmHg, a normal ICP range.

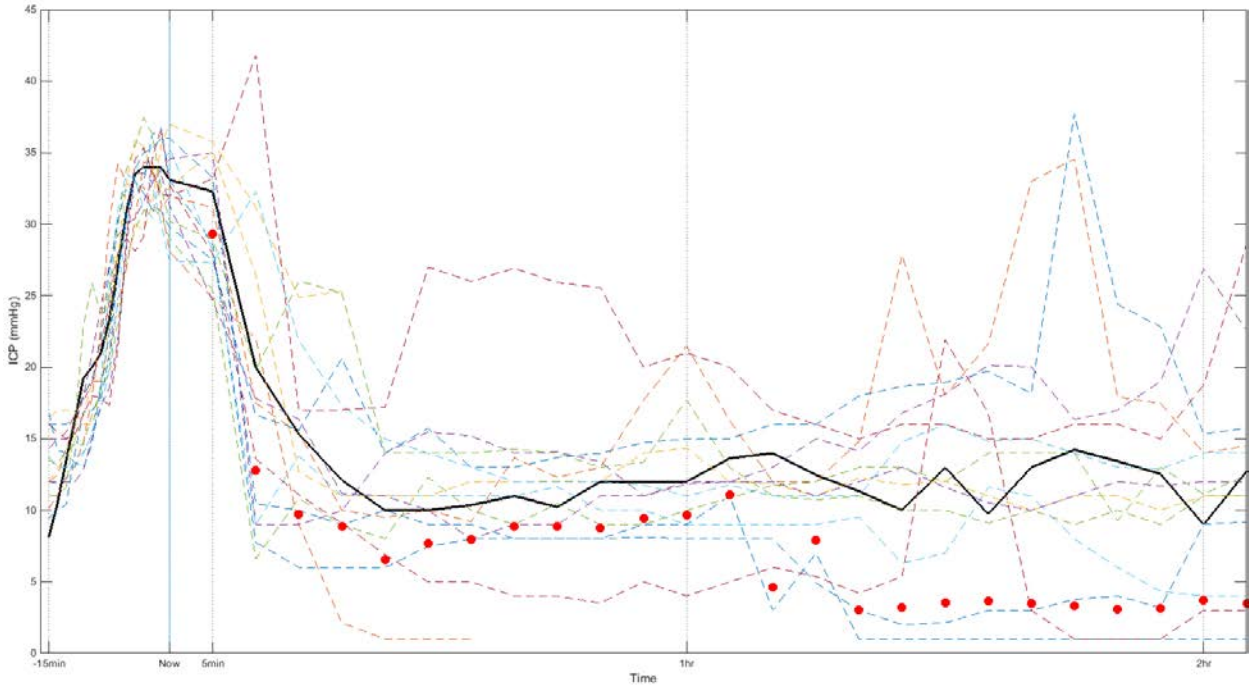


Figure 14. An example shows under-estimated values in the next 2hr.

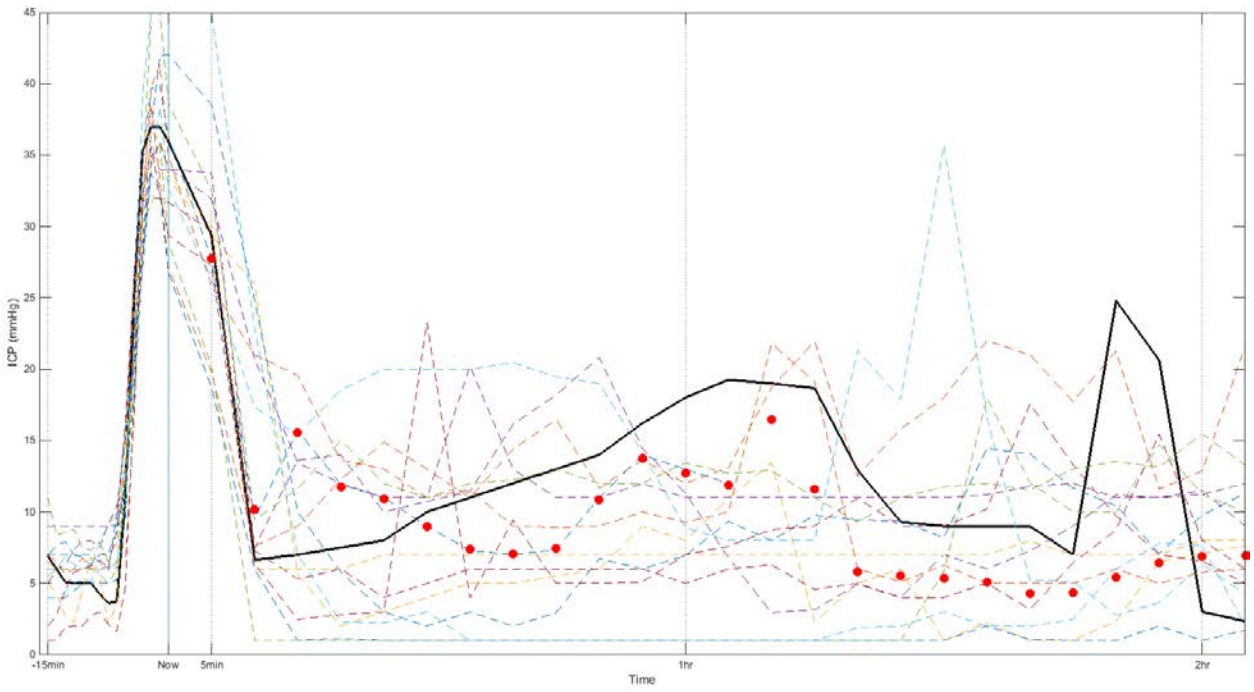


Figure 15. An example shows an estimated episode around 1hr when ICP > 15 mmHg occurred.

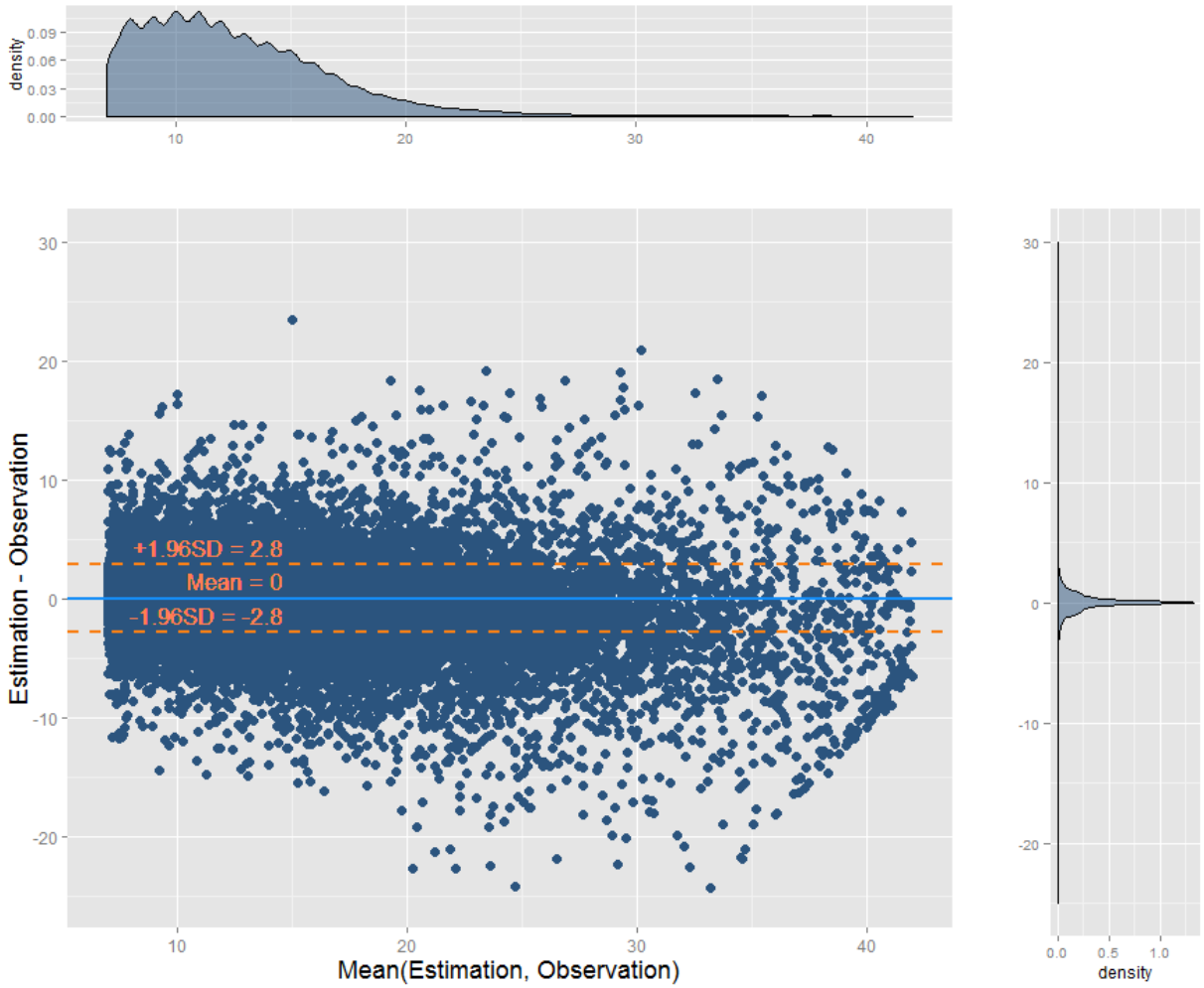


Figure 16. Bland-Altman plot for comparing the predicted 5-minute future ICP values and their observed values. The limit of agreement is 2.8mmHg to 2.8mmHg. The top and right panels show the distributions of data points in the x and y axis.

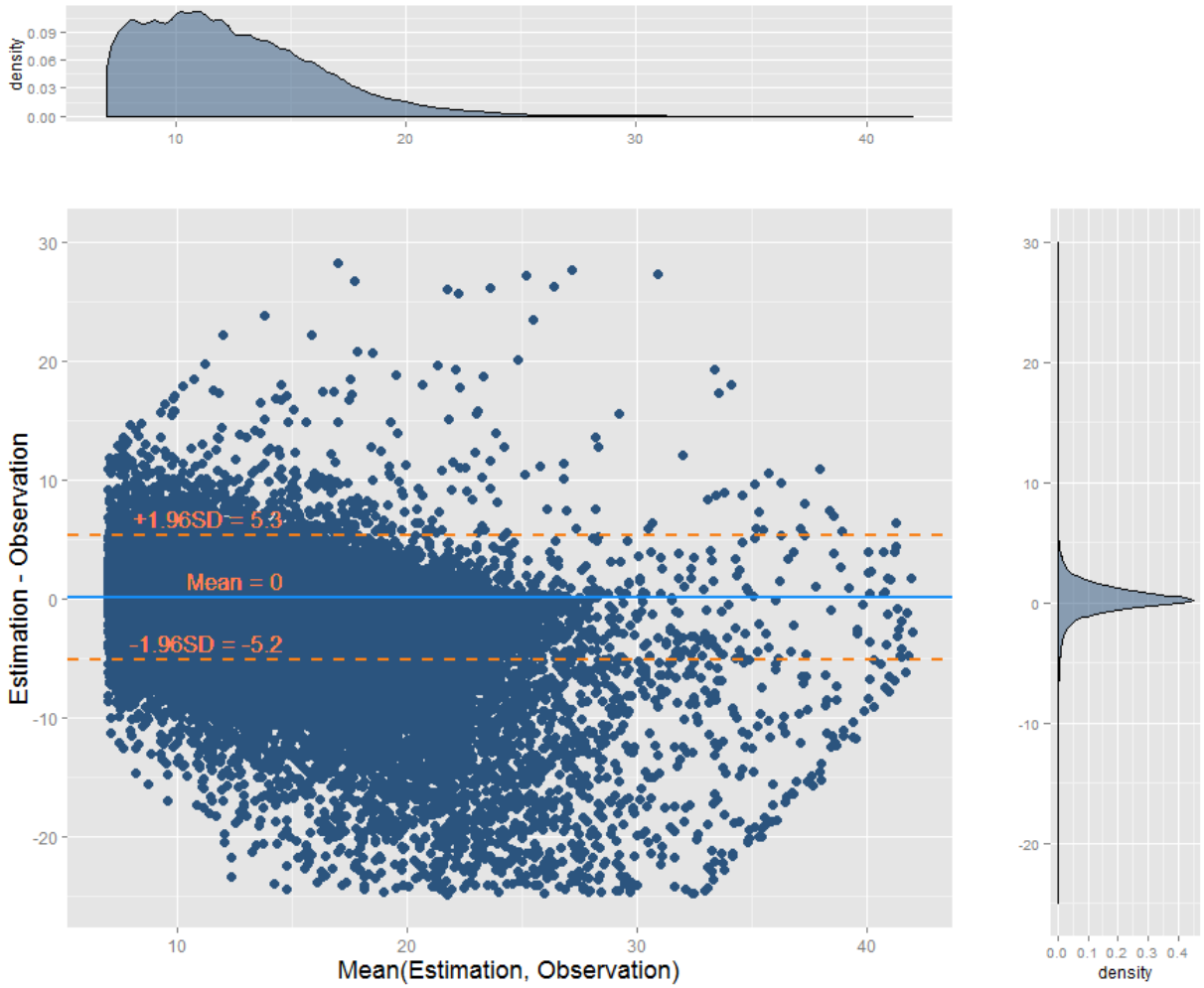


Figure 17. Bland-Altman plot for comparing the predicted 1-hour future ICP values and their observed values. The limit of agreement is 5.3mmHg to -5.2mmHg. The top and right panels show the distributions of data points in the x and y axis.

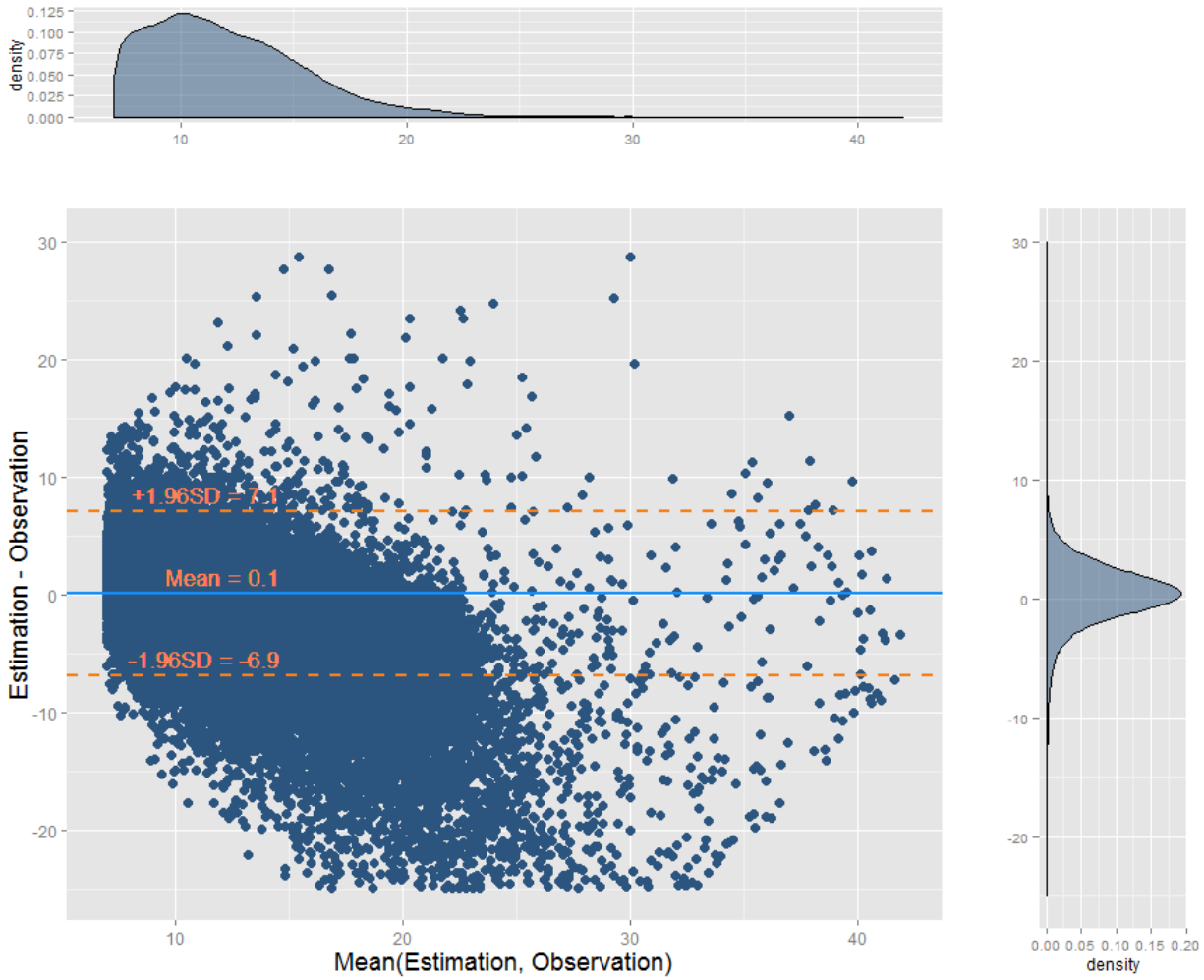


Figure 18. Bland-Altman plot for comparing the predicted 2-hour future ICP values and their observed values. The limit of agreement is 7.1mmHg to -6.9mmHg. The top and right panels show the distributions of data points in the x and y axis.

6.0 DISCUSSION

TBI is a common pathology seen in adult trauma patients. Management of TBI in patients revolves around preventing secondary injury and NW. Early recognition and mitigation of secondary injury could ameliorate the effect of intracranial hypertension and cerebral hypoperfusion, which are known to be associated with worse outcomes [1]. In the military, TBI is a signature injury of the conflicts in the Middle East [2, 3]. A variety of complications that often occur after TBI, including hypotension and hypoxia, can contribute to secondary brain injury and are associated with worse functional outcomes [4, 5, 10]. Many patients experience brief (<30 min) episodes of hypoxia before their hospitalization that may be caused secondary to other injuries or medical evacuation by rotary wing aircraft [4]. Complications from these brief hypoxic episodes include increased neuronal damage, worsened axonal pathology, exacerbated neuroinflammatory response, augmented brain edema, and sensorimotor and cognitive deficits [11-13]. Early prediction of NW could expedite care of critically injured and may improve

patient outcomes. The role of the initial brain CT scan and of repeat brain CTs is well established. Serial CT scans in head trauma are obtained for early capture of neurologic worsening which can lead to early medical and surgical interventions even before the clinical symptoms manifest [6]. Others have recommended relying on clinical examination to analyze the need for a repeat head CT [7-9, 17]. This theory is especially practical in the rural areas, developing countries and combat fields with limited resources. The purpose of the analysis is to predict parameters ahead of time so that clinicians can proactively manage adverse cerebral pressures, leading to improved patient care and outcomes.

It is important to develop predictive models that use features that are elucidated to missing data, a common reality of continuous automated recordings. Our hypothesis was that combining continuous monitoring information, associated with adverse outcome after TBI can be used to predict the likelihood of future onset of adverse cerebral pressures. Our specific aims are to predict the following 4 outcomes in 6 hours subsequent to the time frame of the predictive features. The outcomes include $ICP_{>20mmHg}$ > 30min, $ICP_{>30mmHg}$ > 15min, $CPP_{<60mmHg}$ >30min, and $CPP_{<50mmHg}$ >15min. In addition, biomarker trends for TBI could aid diagnosis, intervention selection and provide vital prognostic information.

Predictive biomarkers for diagnosing TBI have been widely studied. Their potential associations with mild TBI, mortality rate and other long-term outcomes could aid the diagnosis, especially in the situations that invasive monitoring or expensive examination devices are not available. Our data suggests a single biomarker elevation is varied and may not be useful; however, combination of values has the potential to predict a validated model [26]. For instance, IL-10 may have acute and chronic inflammatory marker changes with direct and indirect effect as a biomarker. IL-6 has been shown to be not sensitive enough to detect TBI [27] therefore, a single biomarker elevation may not be attributable. Overall ranking still shows that part of biomarker variables is very important and have strong relations to the outcomes.

For health care in austere environment, diagnosis and prediction models are better to have the capability to use multiple data sources and handle missing values. For example, invasive VS has shown to be useful in predicting near future ICP elevation. However, biomarkers, medication and the combination of other information could act as a surrogate model and provide decision support for the health care provider, when the best predictors are not available. Therefore, creating robust prediction models from the variables that are less predictive but more available could enhance the robustness in the environments such as battle field and natural disaster scenes.

Given the limited number of study subjects, we have to interpret the study results cautiously. This is a single-center study with great potential and has to be tested in a larger population. Animal model studies confirming the validity of biomarker prediction is warranted. Further studies could evaluate our hypothesis by mimicking TBI and air evacuation with hyperbaric exposure in testing innate and adaptive immunity responses. Even without invasive monitoring, predictions about impending elevations in ICP would be possible with the addition of biomarker measurements and could have strong relations to the outcomes. Thus, facilities without ICP monitoring could direct triage of patients and low risk patients could potentially avoid invasive intracranial monitoring and repeated interval monitoring by CT scan.

7.0 CONCLUSION

The brain is thought to be immunologically privileged because of the blood-brain barrier (BBB). However, the breakdown of the BBB after TBI may allow leakage of some humoral factors as well as facilitate recruitment of immune cells. CYT have been correlated to outcomes as well as ongoing, secondary injury progression and the inflammatory component of the complex injury cascade following brain injury may be monitored using different modalities. Using a multimodal monitoring approach can potentially aid in the development of therapeutics targeting different aspects of the inflammatory cascade and improve the outcome following TBI. The leakage of the BBB followed by TBI and a hypoxic event is increased, and this may allow for more factors to cross the disrupted barrier [28, 29]. Increased levels of pro-inflammatory cytokines, IL-6 and IL-8, are seen in human serum after TBI and have been correlated to poor outcomes [15, 16]. In an animal model study, brief exposure to hypoxia after mTBI results in exacerbated brain inflammation and injury. It was noted that systemic neutralization of IL-6 mitigates these effects and reverses loss of motor coordination after mTBI [30].

The neuro-inflammatory cascade following TBI comprises a wide array of both humoral and cellular players. An ever-growing literature has defined changes in cytokine production and cell activation in both the acute and chronic phases following brain injury. However, we are still confronted with the difficulty in placing the individual components into a coherent picture. There is a limit to what we can learn from a single clinical study as to the role of individual mediators/cells and ultimately understand how they affect patient functional outcome.

Our results demonstrated that a component of the inflammatory response in TBI is increased expression of cytokines. The expression of IL-6, as well as in various clinical studies of TBI, is directly associated with the degree of brain injury and outcome [14-16]. By studying multiple cytokines with ICP we were able to identify a combination of biomarkers, which together, provide prognostic data for TBI. Our study was not able to create a biosignature of cytokines, but instead contribute five most important variables containing 3 variable groups. Although the models built entirely from biomarker variables had lower AUROCs than the ones built from invasive VS, this overall ranking still shows that part of biomarker variables are very important and have strong relations to the outcomes. PCA has been suggested as a more accurate method of analysis. We were able to show PCA biomarker patterns could have promising foresight for greater than 24 hours in TBI. In the rural areas, developing countries, and war zones where resources are limited biomarkers have the potential to assist in allocation of resources. Additional investigation into the biomarker patterns may provide additional valuable insights. Further studies to determine the implication for predicting outcomes is warranted. Our data suggests that multimodal statistical analysis may be a rational target for further study of TBI. Finally, future studies may build upon our analysis by using other statistical modeling tools to examine one or more markers and continue to refine algorithms incorporating biomarker levels with physiological data to predict impending events in patients with TBI. Further validation of the algorithms in a larger population is appropriate and necessary. With sufficient historical data and an algorithm, past similar observations could provide reasonable ICP estimations in clinical useful time frames. Further studies would enhance prediction power and could lead to a real-time prediction of neurological worsening.

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APPENDIX

Addendum

Enrollment Timeline/Methods –

The Fit to fly project was IRB approved on January 13, 2013 with a contract award date of May 30, 2013. The approved protocol prospectively enrolled patients of the STC. The overall aim of this study was to investigate the power of inflammatory cytokines and markers of neuronal cell death to predict episodes of cerebral CH, CHx, ICH and the need for neurosurgical interventions earlier and at shorter intervals over the first 3 days after injury than previously described and in a patient-injury population more consistent with combat casualties. Our long-term goal in this work is the fielding of robust and efficacious point-of-care test modalities as adjuncts to "Fit to Fly" air evacuation decision-making.
The initial inclusion/exclusion criteria were as follows –

Inclusion –

- Diagnosis of severe TBI as defined by Head AIS >2 and post-resuscitation motor GCS<6.
- Isolated traumatic brain injury WITH/ OR WITHOUT additional injury as defined by at least one other body region with an injury consistent with AIS >3 OR two body regions with an AIS ≥ 2.
- Placement of a clinically indicated ICP or IVC).
- Patient ≥ 18 years old.
- Enrollment within 6 hours of injury.

Exclusion –

- Patient < 18 years old.
- A non-survivable brain injury.
- An anatomic injury incompatible with survival.
- Patient is pregnant.
- Non-English speaking, consentable party.
- Patient is a prisoner, on parole or probation.
- Patient is on active military duty.

Basic tasks included prospective enrollment on a 24/7 schedule. Appropriate patients would be approached to discuss the study and pursue informed consent. Since this study population required significant brain injury, it was expected that all consents would initially come from the LAR. If a study participant regained sufficient cognitive capacity for consent, then re-consent would be pursued. Clinical data collection including continual vital signs, chart extraction and EMS data was planned. For the cytokine testing, blood sample collection was planned for arrival followed by sampling every 6 hours for the first 72 hours.

An initial meeting was held with the research team on June 13, 2013 to finalize procedures and evaluate the protocol. Initially the study had a 6 hour waiver of consent, which was related to the original inclusion criteria. It was purposed that the waiver of consent be increased to 8 hours to assist with consenting. The blood sample collection and data collection process was finalized. Blood samples would be collected on admission and every 6 hours for the initial 72 hours post admit. Data collection forms were planned and developed. Improvements

to the continuous vital signs system as well as the ICU Team Viewer were initiated. A protocol modification was submitted to the IRB. (Reference FA8650-12-2-6D09 Quarterly Report dated 9/30/13)

On October 12, 2013 a mock enrollment exercise was conducted to test the current protocol. Issues discovered were discussed and revised procedures established as needed. On October 13, 2013 the final installation and testing of the continuous vital signs collection systems was completed. By December of 2013 patient screening and enrollment had been initiated. However, due to multiple logistical delays and reduced patient availability a need to revise the milestone for the enrollment of the first 50 patients was established. By the end of calendar year 2013, 39 patients had been screened and 2 were enrolled. Major protocol modifications by December 31, 2013 include the addition of clinical judgement data points, risk level revised to minimal risk, consent form to indicate follow-up communication and the removal inclusion criteria of enrollment within 6 hour of injury. (Reference FA8650-12-2-6D09 Quarterly Report dated 12/31/13)

By the end of March 2014 more than 120 patients had been screened, 5 eligible subjects were identified and 3 were enrolled. Also by this time it was discovered that the admission blood sample retention and the consent wavier window were not in agreement from a timing standpoint. A protocol modification was initiated to revise the consent waiver window to 12 hours. Enrollment numbers continued to lag behind the original assessments and several team meetings were held to determine potential causes and solutions. Although decent admissions with CT indicating brain injuries were being screened there were additional issues being identified. Several major issues were identified. The requirement of an ICP/IVC being placed within 6 hours, a motor GCS <6 (mGCS), and obtaining consent (from the LAR), within 8 hours generated a difficult enrollment situation that was compounded by an unprecedented low admission cycle at STC. The mGCS score of <6 could not change since it is used to determine the seriousness of the brain injury. The consent window was already being modified to 12 hours, so a decision was made to also modify the ICP/IVC placement window to 12 hours. This modification would only be put into practice if it was determined in the next quarter that enrollment was continuing to be negatively impacted by the 6 hour ICP/IVC placement. Of note, the consenting for the participants in this study was problematic due to the need to get consent from the LAR.

The Monitor of Monitor Systems (MoMS) was developed to view in real-time the continuous vital signs of patients in STC. During this time the MoMS was modified to show ICP outputs in real-time as well. These revisions in MOMS also assisted with improving the tracking of patients within the hospital to (reference FA8650-12-2-6D09 Quarterly Report dated 3/31/14)

Continued enrollment issues resulted in a need to request milestone revisions. By the end of May 2014 a combination of slow enrollment and enrollment "freezes" caused by IRB delays had significantly impacted the Fit to fly project. One enrollment freeze lasted from February 12, 2014 until April 2, 2014. Milestones for enrollment and analysis needed to be revised. At this point it was determined a No Cost Extension (NCE) would be required. From March 1, 2014 to May 31 2014 49 patients were screened, 4 were eligible and 2 were enrolled. Additional data capture requirements were added for vital sign data from the field transport (EMS) as well as hourly medication capture at the bedside. A significant issue was discovered on April 22, 2014. The 6 hour blood specimen collections were not in sync with the standard of care blood collections, thus generating a significant IRB protocol issue (protocol deviation). A new process

was developed for the blood sample collection process. All events and process changes were reported to the IRB. Missing data from the continuous vital signs systems was discovered at this time and it was determined to be caused by cabling issues with the monitors, additional manual collection of data by the research team would be required. On May 14, 2014 the first blood samples were sent to the Cytokine Core Laboratory for initial testing. The MoMS was revised to include vital sign data from the medical chart along with the streamed vitals data coming from the bedside monitors. This revision allowed for periodic validation of data. On May 13, 2014 there were two Request for New Information (RNI) submissions sent to the IRB to detail the protocol deviations on blood sample draws. (Reference FA8650-12-2-6D09 Quarterly Report dated 6/30/14)

From June 1, 2014 until August 31, 2014 the number of potential brain injury patients admitted to STC returned to rates resembling historical norms. During this period 142 patients were screened with 67 having a positive finding of a brain injury. Of those 67 patients 47 either did not receive an ICP/IVC or received one greater than 6 hours from admission. Of the remaining patients, 6 were deemed eligible and 4 were consented for the study. The total enrollment as of August 31, 2014 was 9 patients. The cytokine analysis from the first 3 patients was completed. The MoMS continued to be revised and refined to improve data acquisition and demonstration. New additions to MoMS included a new window to view the cytokine levels over time. (Reference FA8650-12-2-6D09 Quarterly Report dated 9/30/14)

The following quarter of work (9/1/2014-11/30/2014) continued to see a good flow of admissions to STC. During this period 95 patients were screened with 57 being diagnosed with a TBI. Of the 57 patients, 44 did not receive an ICP/IVC or it was not placed within 6 hours of admission. Of the 10 patients that were fully eligible, 3 were enrolled bringing the current study total to 12. By this point in time it was evident that enrollment was so adversely impacted by the startup delays that a NCE of 28 months would be required. The entire period of performance would become 46 months. An additional hurdle to enrollment was the required ICP/IVC placement within 6 hours of admission. Upon further review of all screened patients since the enrollment start date (11/4/2013), it was realized the ICP/IVC inclusion criteria, although valid, inadvertently excluded any patient in which alternate therapeutic treatments were initially undertaken, such as decompressive craniectomy and craniotomy. It also excluded those patients with severe injuries who deteriorated and required monitoring and intervention at a time point beyond the 6-hour window. As a result, a contract modification was submitted to request removing the ICP/IVC criteria in order to be able to capture this valuable TBI patient subset. Although this change resulted in patients being enrolled that did not have data on their intracranial pressures (early in their admission) it would allow for improved enrollment of appropriate patients. This change provided a more complete picture of the entire population of severe TBI patient, thus providing an enriched patient population for viable statistical analysis. On September 2, 2014 the third RNI for Fit to Fly was submitted, which detailed blood sample collection that occurred outside of the 8 hour wavier period granted by the IRB for one patient. During this quarter the cytokine results were received for the next 6 patients enrolled (first 9 patients received at this point). See below for the revised Milestones/Deliverables that were proposed.

Milestones/Deliverables	Status	Revised Estimated Completion Date
All IRB Approvals	completed	January 13, 2013 (actual)
Kick Off	completed	February 8, 2013 (actual)
Contract Award	completed	May 30, 2013 (actual)
Complete installation and testing of cont. data system	completed	October 30, 2013 (actual)
Enroll first 50 patients	pending	September 2015
Complete Enrollment	pending	September 2016
Complete serum analysis	pending	October 2016
Complete statistical analysis	pending	December 2016
Complete scientific reports	pending	March 2017
Complete sponsor reports	pending	June 2017

(Reference FA8650-12-2-6D09 Quarterly Report dated 12/31/14)

From December 1, 2014 to February 28, 2015 several adjustments to the Fit to fly study were requested or put into practice, secondary to prior team discussions on low enrollment and other delays. A 12 month NCE was granted by the Air Force which moved the end of the period of performance to November 28, 2016. At this time it was deemed necessary to pursue an additional extension to complete the established goals. Patient enrollment during this time saw 42 patients screened resulting in 6 eligible candidates with 3 being enrolled. Of the 42 screened patients 28 had TBI, but 21 of the 42 either did not receive an ICP/IVC or it was not placed within 6 hours of admission. The continuous vital sign data for the first 12 patients was completed during this period. On December 3, 2014 a RNI was submitted to document an unannounced onsite Air Force Research Lab (AFRL) audit. (Reference FA8650-12-2-6D09 Quarterly Report dated 3/31/15)

The next quarter (March 1, 2015 to May 30, 2015) saw 58 patients screened with 45 having a diagnosed TBI. Of the 45 with TBI, 25 either did not have an ICP/IVC placed or did not receive it in time. Ultimately, 13 patients qualified and 7 consented for the study. Five of the 13 eligible patients were not able to be enrolled due to the 12 hour window expiring prior to the LAR being located/available. During this quarter the continuous vital sign data for the first 15 patients was completed. RNI number 5 was submitted on April 23, 2015 to inform the IRB of a protocol deviation where a subdural drain was mistaken for an ICP/IVC and the patient was enrolled in the study. Subsequently, once the error was identified the patient was withdrawn from the study (prior to any additional study procedures being done). RNI number 6 was submitted on 5/14/2015 for a patient that was enrolled under wavier, but the LAR eventually declined. Unfortunately, a blood sample was collected out of the waiver window. There were no adverse results for the patient and the LAR was informed. By May 31, 2015 twenty two patients were enrolled in the study. (Reference FA8650-12-2-6D09 Quarterly Report dated 6/30/15)

Significant changes in the Fit to fly study occurred during the quarter of June 1, 2015 to August 31, 2015. Secondary to the slow enrollment and the need to revise the study population, the inclusion criteria was modified. The revised criteria would remove the requirement for a patient to receive an ICP/IVC within 6 hours of admission as well as the revision of "Non-survivable brain injury" declaration. The revised criteria were approved by the UMB IRB on June 24, 2015 and by the AFRL IRB on July 29, 2015. Once IRB approvals were secured enrollment could be reinitiated. This change not only increased study enrollment potential, but

also included any patient in whom alternate therapeutic treatments were initially undertaken, such as decompressive craniectomy and craniotomy and/or drug therapies. These eligibility criteria changes also allow the inclusion of patients with severe injuries who deteriorated and required monitoring and intervention at a time point beyond the 6-hour window. As a result, we were able to capture the "need to fly" TBI patient subset - patients in need of urgent neurosurgical interventions requiring an evacuation to advanced level care. Following the revision of the criteria a new modification to the statement of work (SOW) was drafted and prepared for submission. The revised SOW included the retrospective enrollment of a minimum of 50 participants who meet all eligibility requirements and protocol definitions. A draft of the IRB protocol for the retrospective portion was submitted for IRB approval during the following quarter. The protocol tentatively included the retrospective enrollment of up to 200 patients diagnosed with severe isolated TBI (Head AIS >2 and post-resuscitation mGCS <6) with or without additional injuries from July 2013-September 2015. Retrospective chart review included continuous electronic automated vital signs and physiologic parameters, intracranial pressure, cerebral perfusion pressure (CPP), pressure-times-time of ICP and CPP, shock index (systolic blood pressure divided by heart rate), Brain Trauma Index (CPP/ICP), hourly nurse recorded ICP, and outcome data. The purpose of the retrospective portion of the study was to validate the power of previously developed algorithms to predict long-term functional outcomes and mortality after severe TBI using novel, retrospectively required continuous ICP and vital sign monitoring data, and to predict the severe TBI patient ICP hypertension up to 6 hours into the future. This quarter experienced two different screening periods, one with the original criteria and one with the revised criteria. Overall 68 patients were screened with 19 being eligible and 4 eventual enrollments. Of the 4 enrolled patients this quarter, 2 patients received a craniectomy or craniotomy within the first 6 hours of admission, but received an ICP monitor more than 6 hours after admission. These patients would have been ineligible with the original study criteria. During this period the vital sign data was completed through patient number 22 and cytokine testing was performed on the samples for the next 15 enrolled patients. (Reference FA8650-12-2-6D09 Quarterly Report dated 9/30/15)

The first full quarter with the revised enrollment criteria saw a dramatic increase in enrollments. From September 1, 2015 through November 30, 2015 there were 93 patients screened with 63 having a diagnosed TBI and after other criteria were applied 28 remained eligible. Of the remaining 28 patients 14 were enrolled. LARs not being available within the 12 hour waiver window eliminated 13 of the 28 and 1 LAR declined participation. Data collection continued, as in previous quarters. Bedside manual data collection continued as a "stop gap" for any issues with clinical documentation or automation via the continual vital signs systems. By this point there were 40 patients in the data collection process. Of the 40 patients 36 had completed bed tracking and 24 had all required information collected. On October 28, 2015 the revised SOW (retrospective enrollment) was submitted to the UMB IRB. The UMB IRB approved the retrospective review on November 16, 2015. The AFRL IRB was then sent the revised SOW for approval. This request to revise the SOW caused the Milestone/Deliverable of IRB approval to be revised to "pending" for reporting purposes. Additional requested changes to the protocol included adding collection of the Extended Glasgow Outcome Scale (GOSE) via phone to the informed consent form. (Reference FA8650-12-2-6D09 Quarterly Report dated 12/31/15)

From December 1, 2015 through February 29, 2016 a total of 65 patients were screened with 44 being diagnosed with a TBI. After additional criteria were applied 21 remained eligible.

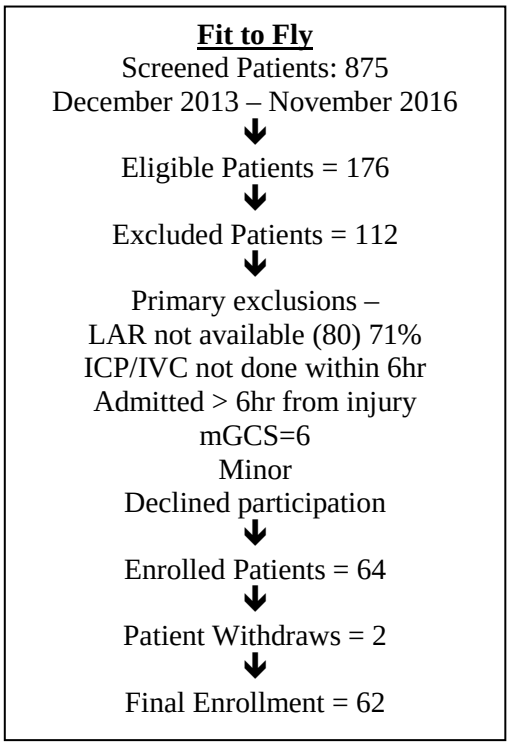
Of the remaining patients 9 were enrolled, 10 had LARs that were not available within the 12 hour waiver window and 2 LARs declined participation. The retrospective SOW revision continued to be pending with the AFRL IRB during this period. With sufficient serum samples (250) and vitals data initial model building was initiated for the first 24 enrolled patients. Additional analysis was initiated at this time on the data from the first 39 patients (reference FA8650-12-2-6D09 Quarterly Report dated 3/20/15)

The higher enrollments continued in 2016 with 58 patients being screened, 22 being eligible and 8 being enrolled in the period from March 1, 2016 to May 31, 2016. Collection of the Extended Glasgow Outcome Scale (GOSE) follow-up data continued in clinic and by phone. Data analysis progressed with the analysis of 39 enrolled patients where they were evaluated on the ability to predict neuroworsening (NW) by using noninvasive vital signs. CT performed at 0, 6, and 24 hours post admission were reviewed. When compared to baseline CT, NW at 6 and 24 hours was diagnosed using the variables: cerebral contusion, cerebral ischemia, compression of basal cisterns, and midline shift. Physiologic variables collected between 0-6 hour and 6-24 hour time frames were used to predict NW diagnosed on CT at 6 and 24 hours. Compared to baseline, 21/75 interval scans showed NW. A model using 3rd quartile SBP and 2nd quartile end tidal carbon dioxide (EtCO2) was able to predict NW with an Receiver Operating Characteristic curve (ROC) of 0.83 and 0.78 for testing set. The model helped in predicting NW in trauma patients with TBI and helped stratify the risk. Low risk patients could avoid invasive intracranial monitoring and repeated interval monitoring by CT scans. The pilot study demonstrated great potential but necessitates validation in a larger cohort. Analysis during this quarter included reviewing 50 enrolled patients, evaluating the ability to predict NW in TBI patients using noninvasive vital signs. Baseline head CT performed at the time of admission and interval CT scans at approximately 6 hours and 24 hours post admission were reviewed. Interval CT scans were compared to baseline CT using 4 variables – cerebral contusion, cerebral ischemia, compression of basal cisterns and midline shift. The comparison resulted in 3 possible outcomes – stable, worsened or improved on the interval CT. Physiologic variables collected up to 6 hours prior to the interval CT scans were used to predict NW diagnosed on interval CT. Heart rate, respiratory rate, systolic, mean and diastolic blood pressure (S/M/DBP), end tidal carbon dioxide (EtCO2), 1st, 2nd and 3rd quartile (1Q, 2Q, 3Q) of the above vital signs and varied thresholds for each vital signs were used in the multivariate logistic regression model for outcome prediction. Receiver Operating Characteristic curve (ROC) and leave-one-out cross validation were used to evaluate the predictive models. The model was able to predict interval NW with a ROC of 0.76 ($p<0.05$) when using 1-6 hour physiologic data prior to the interval scan and with an ROC of 0.70 ($p<0.05$) using 1 hour physiologic data prior to the interval scan. The results of this analysis were submitted as an abstract to the 2016 Military Health Research Symposium (MHSRS). (Reference FA8650-12-2-6D09 Quarterly Report dated 6/20/15)

From June 1, 2016 to August 31, 2016 there were 65 patients screened and 32 were deemed eligible. LARs were not available within the wavier window for 13 patients and 7 LARs declined. Other criteria excluded and additional 7 leaving 5 enrolled. There was one patient withdrawn during this quarter secondary to the LARs family wishes. The AFRL IRB approval for the retrospective study that was submitted on October 27, 2015 was approved on August 11, 2016. The Milestone/Deliverable for IRB approvals was once again complete. With this approval the team initiated work on the retrospective study by querying the STC trauma registry and collecting applicable patients and data. (Reference FA8650-12-2-6D09 Quarterly Report dated 9/20/15)

By the end of November 2016 a couple significant Milestones/Deliverables were completed. Prospective enrollment of patients was completed with a total of 62 patients being enrolled. In this quarter (September 1, 2016-Novenber 30, 2016) 13 patients were screened with 10 being eligible. Only one was enrolled after 7 LARs were not available in the waiver window and 2 LARs declined to participate. The blood sample collection was completed for the prospective enrollments and the final samples were shipped to the cytokine lab for processing. The Milestone/Deliverable for interim analysis of the prospective patients was also completed. The interim analysis of the first 24 enrolled patients with cytokine results being available resulted in the 3 STINFO cleared abstracts that were submitted to the American Association for the Surgery of Trauma (AAST) and American College of Surgeons 2016 Clinical Congress. Work on the retrospective study continued with patient enrollment and data collection. (Reference FA8650-12-2-6D09 Quarterly Report dated 12/20/15)

Enrollment review – From December 2013 to November 2016, 875 patients were screened for Fit to Fly. Screening was interrupted and put on hold several times secondary to IRB delays. From the 875 patients screened 176 were identified as eligible. Of the eligible patients, 64 (37%) were enrolled from the 176 eligible. Ultimately, 2 patients withdrew from the study leaving 62 total enrollments. Of the remaining 112 eligible patients, 80 (71%) were not enrolled due to a LAR not being accessible within the waiver window for informed consent. The remaining patients (32) were excluded for criteria like not receiving an ICP/IVC within 6 hours (early criteria), admitted greater than 6 hours from time of injury, having a mGCS=6, being a minor or declined participation.



LIST OF ABBREVIATIONS AND ACRONYMS

AUROC	area under the receiver the ROC curve
BBB	blood-brain barrier
BTF	brain trauma foundation
CH	hypoperfusion
CHx	cerebral hypoxia
CPP	cerebral perfusion pressure
CT	computed tomography
CYT	cytokines
GCS	Glasgow Comas Scale
ICH	intracranial hypertension
ICM	intracerebral monitoring
ICP	intracranial pressure
IL-8	interleukin 8
IRB	institutional review board
MoMS	monitor of monitor systems
NIH	National Institute of Health
NNR	near neighbor regression
NW	neurological worsening
PbO_2	brain oxygenation
PCA	principal components analysis
STAR-ORC	Shock Trauma and Anesthesiology Research-Organized Research Center
STC	R Adams Cowley Shock Trauma Center

SVS	systemic vital
TBI	traumatic brain injury
TRU	trauma resuscitation unit
UDSOM	University of Maryland School of Medicine